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Arthropod succession on rat carrion in West Virginia

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Carcasses represent a temporary and changing food source for a varied, yet distinct, community of organisms. Arthropods are the major component of this community and are the driving force for the early decomposition process. Although carrion remain for only a finite time, they represent an ecosystem which has a distinctive faunal succession usually initiated by calliphorid flies and variously followed by staphylinid, histerid, and dermestid beetles (Reed, 1958; Payne, 1965; Greenberg, 1991). The developmental rate of arthropods and their faunal progression is being increasingly used in determining time or site of human death. The most widely used application of arthropods in forensics is in determining post mortem interval (PMI). Since most bodies are discovered within the first few weeks, the insects of primary importance in forensic entomology are the blowflies (Calliphoridae) and the flesh flies (Sarcophagidae), which arrive when death is anticipated (Davis, 1928) or immediately after death.

The use of insects to aid forensic investigations is not a new concept. The role of necrophagous insects was understood even in biblical times with specific references to carrion insect activity in Job: 21:26, 19:26, 24:20, and Isaiah: 14:11 (Byrd, 1995). Additionally, even the fly life cycle was understood by ancient Egyptians. A piece of paper found in the mouth of a mummy stated: "The maggots will not turn into flies within you" (Papyrus Gizeh no. 18026:4:14) (fide Greenberg, 1991). A

13th century Chinese manual on forensic medicine, published in 1235 AD entitled *The Washing Away of Wrongs* (as translated by McKnight, 1981), records how one investigator used insects to solve a murder in a farm community. When farmers were assembled with their sickles, the presumed murder weapon, flies clustered on one sickle, in particular, probably due to the traces of the victim's blood. Once confronted, the owner of this sickle confessed (McKnight, 1981).

Forensic entomology did not emerge as a science until the mid 19th century when the French pathologist Orfila (1848) first listed 30 insects and other arthropods that visited a human corpse to feed and oviposit (Greenberg, 1991). Although Orfila was the first to examine insect activity on a human cadaver, Bergeret (1855) was the first to apply this knowledge of arthropod succession on a human corpse. Megnin (1894), the founder of forensic entomology, identified eight stages in the decomposition of a human body on land and recorded the insects associated with each stage. However, his paradigm of succession has proven to be somewhat of a hindrance, in that strict adherence to a timetable of arrivals and departures of various carrion-feeding species is misleading in estimating PMI (Erzinclioglu, 1983).

Faunal succession has been studied in various geographic regions in non-human cadavers. Payne (1965) studied arthropod activity on the baby pig, *Sus scrofa*, in South Carolina while Reed (1958) observed arthropod succession on dog carcasses in Tennessee. Other recent

faunal succession studies have been conducted in diverse habitat types ranging from sand dunes in Northern France to tropical rainforest and island habitats (Richards and Goff, 1997; Tomberlin and Adler, 1998; Bourel et al., 1999; DeJong and Chadwick, 1999). These studies provide useful information on community structure, colonization order and seasonality for those arthropod species of forensic interest in the geographic areas examined.

In recent years, the field of forensic entomology has undoubtedly experienced a renewed interest. This may be due, in part, because the precise time of death is generally very difficult to estimate after the decomposition process has been underway for more than 24 hours (Kaplan, pers. comm., 1999). Toxological analysis of the insects feeding on cadaver tissue can demonstrate the presence of ante mortem drugs and toxins when suitable blood, urine, or tissue is lacking (Nuorteva and Nuorteva, 1982; Gunatilake and Goff, 1989; Goff et al., 1991; Goff et al., 1992; Goff et al., 1993; Byrd, 1995). Then too, murder victims are sometimes moved from the scene of the act to a different geographic setting. Forensic entomology may play a significant role in these instances when certain species of insects found in the final resting area of the corpse are not compatible with insects found in the corpses' tissues or clothing (Lord et al., 1986a, 1986b; Byrd, pers. comm., 1999). For example, *Cynomyia mortuorum* is confined to littoral habitats along the coast of France and can be used as an indicator of movement of a body

following death (Bourel et al., 1999). Since arthropods have proven to be so useful in helping solve murder cases, it is important to study their development rates and colonization on carrion in field conditions.

The current project was undertaken for two reasons. The first is that virtually nothing has been published relative to arthropods that visit carrion in West Virginia. Thus, the goal here is to identify some of those species, especially flies and beetles, that are endemic to the state. The second reason is that we know little about development rates of carrion flies under field conditions. Thus, the second goal of the study was to ascertain the development of fly life cycle stages over periods of 11 days in four different seasons. The current project examines colonization of rat carcasses on land in shaded versus sunlit conditions, since Reed (1958) observed that carcasses in pasture areas had smaller insect populations as compared to those in wooded areas. Past studies have indicated more rapid maggot development and succession in pasture areas than those in wooded areas (Cole, 1942; Reed, 1958; Erzinclioglu, 1986). Arthropod succession on male versus female carrion is also addressed.

CHAPTER 2

FLY DEVELOPMENT

Development has been considered only for two groups of insects in this study; the calliphorid flies (Calliphoridae) and sarcophagid flies (Sarcophagidae). There are two reasons for this: 1) it would be impractical to cover developmental stages of all arthropod groups involved in a single study, and 2) the flies are the primary macro-decomposers in the early decay stages of carrion in a natural setting (Williams, 1984).

Calliphorid Development

The term "calliphorid" is commonly assigned to flies placed taxonomically in the family Calliphoridae. Flies of this family have acquired additional common names: carrion flies because of their larval feeding habits; green-bottle or blue-bottle flies because of the iridescent coloration that is characteristic for the adult abdominal region; or blowflies because of the rapid egg-laying (i.e., oviposition) techniques employed by the female fly.

Blowflies have a keen ability to locate ephemeral habitats (e.g. carrion, garbage, decaying animal wastes) in large areas. Calliphorids are extremely olfactory sensitive and can move 20 km in a day, although they probably quest over greater distances in open country (Greenberg, 1991). Mono and polyamines that arise as decarboxylation products of amino acids are thought to be among the important chemical attractants to

carrion (Erzinclioglu, 1986). If environmental conditions are favorable, the female blowfly lays approximately 250 eggs, often in a batch. The optimum oviposition temperature for the adult ranges from 15 – 25 °C (Smith, 1986). Thus, the forensic application of blowflies begins with the egg; however, it is the least important stage since eggs usually hatch quickly (i.e., 18-24 hours), often before a body is discovered. Lowered temperatures, and even elevated temperatures (i.e., > 37.5 °C), can prolong egg hatch times (Melvin, 1934).

Predators of carrion fly eggs include staphilinid, silphid,and histerid beetles, and ants (Reed, 1958; Payne, 1965). Perhaps the mounding of eggs, sacrificing only the eggs at the periphery of the batch, lowers the risk of egg predation. Risk of egg predation is further reduced by brief incubation periods.

First instar larvae (i.e., maggots) emerge from the eggs and further develop into second and then third instar larvae; the last instar being used most frequently for identification purposes because of its larger size and more heavily sclerotized (i.e., hardened) body structures. These feeding aggregations of growing larvae are extremely exothermic. Deonier (1940) measured maggot mass temperatures in sheep and goat carcasses in winter and noted that, "as larval development progressed, the temperature increased and remained high regardless of daily weather fluctuations." When ambient temperature was 9 – 22 °C, he recorded a maximum maggot mass temperature of 49 °C. The maggot mass remained active

even when the air temperature reached – 4 °C. Thus, as a result from their exothermic nature, actively feeding maggots can develop fairly rapidly even in cold weather conditions. Once post-feeding larvae disaggregate, the maggot mass temperature falls rapidly (Greenberg, 1991).

Growth rate changes due to the metabolic heat generated by their active feeding must be considered when estimating the PMI. Developmental rates determined from "constant" laboratory temperatures must be used with caution since temperatures can be significantly elevated in second and third larval instars. Then too, developmental times derived from "constant" temperature laboratory rearing can differ significantly from larvae exposed to cyclic temperatures found under natural conditions (Greenberg, 1991). Thus, recent work has focused on the effects of temperature on larval development (Byrd and Butler, 1996; 1997; 1998).

From an evolutionary standpoint, rapidly developing eggs and feeding larval stages are selected for due to the fleeting nature of the carrion habitat. In fact, the post-feeding larva and pupa account for approximately 75% of total pre-adult time (Greenberg, 1991). Once reaching the post-feeding (wandering) stage, massive numbers of maggots exit the carcass in search of a suitable pupariation site. Once the maggot stops feeding, it wanders for about 4 days (at 22 °C) or 3 days

(at 29 °C) and some distance away from the carcass before it burrows into the ground for pupariation (Greenberg, 1991).

During pupariation, the soft white larval cuticle is transformed into a rigid, dark puparium, which includes shortening and broadening of the larva. Respiratory horns puncture the cuticle and make contact with the exterior once sclerotization is complete (Greenberg, 1991). The next major development is the pupal-adult apolysis. At this point the white pharate adult is enclosed within the pupal casing. Pigmentation of the compound eye begins at 81 hours after pupariation and is completed by 88 hours (Greenberg, 1991). Beginning at 120 hours at 22 °C and ending at 134 hours, tanning of the setae on the head and thorax occurs (Greenberg, 1991). These transformations, which follow a distinct time line, are useful in indicating ages of flies for forensic investigations.

Sarcophagid Development

Adult sarcophagid flies, sometimes called flesh flies, are placed in the family Sarcophagidae. Adults of this family are gray/black flies characterized by 3 black longitudinal stripes on the thorax and a "checker-board" design on their abdomens. Sarcophagids are unique in that the females do not lay eggs, but rather deposit living first stage larvae on carrion. Beyond that, larval development and pupariation (sometimes called pupation) is, in general, like that described previously for the calliphorids.

CHAPTER 3

DESCRIPTION OF STUDY SITE

The present study was conducted at Green Bottom Wildlife Management Area (GBWMA), a 338-hectare area adjacent to the Ohio River in Cabell County, West Virginia (U. S. Geological Survey Topographic Map; Athalia Quad, Military Grid Reference Point ^{3}9050; ^{42}7209; elevation = 550 ft) (Figs. 1, 2, and 3). This area was purchased and established by the Corps of Engineers in the late 1980's to mitigate for fish, wildlife, wetland, and public losses resulting from the Gallipolis Locks and Dam Replacement Project located some 18 km upstream on the Ohio River. A mitigation plan was jointly developed by the West Virginia Department of Natural Resources (WVDNR), the U. S. Fish and Wildlife Services, and the Corps of Engineers. This plan was forwarded to Congress in the December 1980 Fish and Wildlife Coordination Act Report (WVDNR, 1989). Wetland habitat was expected to double under this agreement along with an accelerated wetland plant succession due to the planting of buttonbush, silky dogwood, and smooth alder (WVDNR, 1989).

The Report of the Chief of Engineers, dated April 8, 1982, and the Supplemental Report of the Chief of Engineers, dated August 13, 1983, included a recommendation to purchase, enhance, and manage the Green Bottom Swamp (i.e., GBWMA) area. The Green Bottom Swamp was identified by the report as the; "most feasible location: for necessary acquisition of mitigation lands"; and the report recommended such lands

be acquired "...to fully mitigate all remaining project wildlife losses..." (WVDNR, 1989). The Draft and Final Environmental Impact Statements, which include the Fish and Wildlife Coordination Act Report with its management recommendations, were distributed to national, state, and local organizations as well as federal, state, and local officials (WVDNR, 1989). Congressional approval and federal funding were obtained after considerable review of the project plans. The WVDNR signed a 25-year lease with the Corps of Engineers on February 20, 1989 for the management of the GBWMA mitigation lands as a state hunting and fishing area (WVDNR, 1989; Hamrick, Fagan, and Farewell, pers. comm., undated, Appendix I).

The GBWMA is located 19.6 km north of Huntington, West Virginia (Allen et al., 1995). The majority of the area is located between State Route 2 and the Ohio River in Cabell and Mason Counties (Fig. 1). Along the northern border of GBWMA, the Ohio River shoreline extends 14,450 ft (WVDNR, 1989). The GBWMA contains the following vegetative classifications: forestlands, 162 acres; wetlands, 140 acres; agricultural land, 518 acres; and open water, 16 acres (WVDNR, 1989). Cropland and pasture exist south of State Route 2 and cover over 75 percent of the area.

Bottomland hardwoods dominate the forestlands located at GBWMA (Stark, 1993). Silver maple, sycamore, cottonwood, and boxelder are the most abundant trees along the Ohio River. Slippery elm,

river birch, green ash, yellow-popular, black cherry, black walnut, and black willow are the other tree species found in less abundance. Major woody understory components include black willow, multiflora rose, and ironwood (WVDNR, 1989).

Four wetland types compose 58 ha of GBWMA and include the following: seasonally flooded flats, inland open fresh water, shrub swamp, and wooded swamp (Allen et al., 1995). The Green Bottom Swamp, which encompasses about 100 acres, is located near the center of GBWMA. Homestead Creek, which is surrounded by a narrow bottomland hardwood forest, drains the swamp. A 25 acre shrub swamp and a 1 acre wooded swamp, located 14.3 km east of Lesage, West Virginia, are situated upstream and downstream (Ohio River) of Green Bottom Swamp, respectively (WVDNR, 1989).

CHAPTER 4

MATERIALS AND METHODS

Field Methods/Experimental Design

Four experimental periods were selected to assess the species of carrion flies, beetles, and miscellaneous taxa that visit carcasses at different times of the year. Each experimental period covered a span of 11 days (Day 0 through Day 10) with experimental periods I, II, III, and IV conducted in March/April, May, July, and October, respectively. Specific starting and ending dates for each experimental period are shown in Table 1.

Two experimental plots were established for each experimental period: one in sunlit (field) conditions and the other in shaded (forest) conditions. Figure 2 A-C shows plots in March when there were no leaves on the trees and Figure 3 A-D is of plots at a time when trees displayed a full leaf canopy. Climatological data for each plot (sunlit and shaded) in each of the four experimental periods are presented in Table 2.

Each plot was designed so that 11 shallow depressions (50 cm long X 30 cm wide X 15 cm deep) were dug into the ground in a circular fashion. The diameter of this circular design was 7 m, with the centers of each depression placed 2 m from each other (Fig. 4 A-B). Plastic containers were snugly fitted into each depression; their upper rims flush with the ground surface. Four holes, each with a diameter of approximately 1 cm, were drilled through the bottom of each plastic

container to allow for water to escape the containers and to provide access for those arthropods (chiefly beetles) seeking entrance from underground.

Twenty-two white male Sprague-Dawley outbred rats (*Rattus rattus*), weighing from 275 to 300 g, were killed by oxygen deprivation and cervical dislocation in the laboratory. The weight of each rat was verified to the nearest 1.0 g on an Ohaus® portable electronic balance, Model BB300. Rats were then transported to the GBWMA site immediately after weighing. A single rat was placed in each of the 11 sunlit and 11 shaded plastic containers after an incision had been made from immediately anterior of the testis to the base of the rib cage, exposing the abdominal viscera. Rats were placed on their left sides with heads pointing in a clockwise direction and abdomens directed toward the center of the circle (Fig. 5 A). After all rats had been appropriately positioned in their respective containers, the containers were covered with 2.5 cm hexnetting and secured with metal stakes to exclude predators/scavengers (Fig. 5 B).

Experimental period II differed from the remaining periods in that both male and female rat carrion were used to evaluate whether or not carrion fly colonization differs based on the sex of the carrion. Thus there were three experimental plots—two in shaded conditions (forested), one in sunlit conditions (field)—for experimental period II. A total of 33 white Sprague-Dawley outbred rats, weighing from 275 to 300 g, were utilized; 22 male and 11 female. A single male rat was placed in each of the 11

sunlit plastic containers and similarly placed in each of the 11 shaded plastic containers of one of the two shaded experimental plots. In the remaining shaded experimental plot, a single female rat was placed in each of the 11 plastic containers.

The following environmental conditions were recorded daily at both the sunlit and shaded plots: ambient temperature (hand-held thermometer), ground temperature (hand-held thermometer), soil temperature at a depth of 10 cm (hand-held thermometer), precipitation (rain gauge), and relative humidity (sling psychrometer) (Table 2). Ambient temperatures (°C), collected daily at the site (both sunlit field and shaded forest), were graphed along with daily maximum and minimum ambient temperatures acquired from the National Weather Service recording station located at Lesage, West Virginia (Fig. 6).

Field Methods/Carrion Fly Collections

The day of rat placement was designated as Day 0 (Day Zero) for each experimental period. Rats were placed in their respective plots (i.e., sunlit and shaded) at approximately noon on Day 0 of each experimental period. On Day 0 plus 2 h of experimental period I, one rat from the sunlit and one from the shaded plot was removed from its respective sunlit and shaded container. Each of these two rats was placed in separate 1 gal plastic zip-lock bags and returned to the laboratory. Upon arriving at the laboratory, the rats were removed from their respective bags and

examined for the presence of carrion fly eggs and/or larvae. This procedure was repeated daily (i.e., for Day 1 through Day 10) in a clockwise manner (Fig. 4 A) over the next 10 days, after which all rat carcasses from each plot had been examined. Rat placement and collection for the examination of fly eggs and larvae were repeated in a similar fashion for all days of subsequent experimental periods (i.e., periods II, III, and IV). The only deviation from this procedure occurred in experimental period II, which involved the collection and examination of two rats (1 male and 1 female) from shaded conditions. Intact rat carcasses (Fig. 7 A-B) were returned to the laboratory in zip-lock bags; however, it should be noted that after a few days, rat carcasses had disintegrated (depending upon weather conditions) to such a point that it was impractical to remove the carcass intact (Fig. 7 C-E). In the latter case, a sample of fly eggs and larvae were removed with a scoop, placed in an appropriately labeled 250 ml screw-cap bottle and returned to the laboratory.

Fly larvae, whether conveyed to the lab with a rat in a zip-lock bag or in a bottle, were killed within 30 min after arriving at the lab by immersion in boiling water. Larvae were then fixed and stored in 10% buffered formalin in appropriately labeled bottles (i.e., noting the experimental period, day of collection, and sunlit or shaded conditions). This procedure provided a sample of fly larvae for each day of each experimental period in which larvae were present. From these samples,

information could be gained on the species of flies colonizing carcasses and the development of fly larvae on a daily basis for each of the four experimental periods.

Adult flies were captured from the site by sweep net for identification or were simply identified as they rested on the carcasses at the site. Selected fly larvae were reared on beef liver to the adult stage in the laboratory to confirm species level identifications.

Preserved third stage fly larvae were prepared for identification by removing the anterior segments containing the cephaloskeleton and anterior spiracles and preparing the posterior-most region of the larvae so that the posterior spiracles could be viewed in a cross-sectional aspect. Such tissue preparations were dehydrated in an ethanol series, cleared in xylene, and mounted on glass slides in Canada balsam. Care was taken to ensure that each cephaloskeleton was matched with its respective set of posterior spiracles (i.e., to avoid the possibility of placing the cephaloskeleton of one species on a slide with the posterior spiracles of a different species). Third stage larvae were identified based on pictoral keys (CDC, 1967) and the keys provided by Hall (1948). Adult flies were keyed using Hall (1948).

Field Methods/Beetle Collections

Each container containing a carcass in both experimental plots was examined daily for the presence of beetles. All living beetles found

associated with the carcasses were recorded and their relative abundance (i.e., number of individuals per species) was noted daily. Specimens that could not be immediately identified at the site were captured and taken to the laboratory for proper identification using a coleopteran key (Poole and Gentili, 1996). A reference collection was maintained.

Field Methods/Miscellaneous Taxa

The presence of all other macro organisms (i.e., millipedes, centipedes, ants, yellow jackets, pill bugs, snails, and slugs) observed associated with the carrion was noted daily; however, of these taxa, only millipedes, centipedes, and slugs were collected. Millipedes and centipedes were preserved in 10% buffered formalin. Selected slug individuals were relaxed in menthol (1 part saturated menthol solution: 100 parts water) for approximately 2 hours. After relaxation, slugs were killed by immersion in boiling water and preserved in 10% buffered formalin.

CHAPTER 5

RESULTS

A total of 43 species were observed throughout the four experimental periods; 1 centipede, 2 millipedes, 27 coleopterans, 7 dipterans, 1 hemipteran, 2 hymenopterans, 1 isopod, and 2 stylommatophoran molluscs (Table 3).

MYRIAPODS (CHILOPODS AND DIPLOPODS)

Myriapods were found in association with the carcasses only during experimental period I (Tables 1 and 3). The three species observed were the centipede *Scolopocryptops sexspinosus* (Say) and two species of millipedes, *Ophyiulus pilosus* (Newport) and *Pseudopolydesmus* sp. (Table 3). *Ophyiulus pilosus*, a distinctly black species, was clearly the dominant form being collected daily from Day 1 through Day 10, whereas *Pseudopolydesmus* sp. was only observed on 3 separate days (Table 4). Additionally, a total of 225 *O. pilosus* individuals were collected (220 from carrion in the forest plot and 5 from carcasses exposed to sunlit conditions) whereas only 4 *Pseudopolydesmus* sp. individuals (3 from forest carcasses and 1 from the sunlit plot) were observed (Table 4).

INSECTA (DIPTERANS)

Fly species recorded throughout the four experimental periods include the following: *Cynomyopsis cadaverina* (Robineau-Desvoidy) (Fig.

8 A-B), *Phaenicia caeruleiviridis* (Macquart) (Fig. 9 A-C), *Cochliomyia macellaria* (Fabricius) (Fig. 10 A-D), *Musca domestica* Linnaeus, *Fannia canicularis* (Linnaeus), *Sarcophaga bullata* Parker (Fig. 11 A-C), and *S. haemorrhoidalis* (Fallen) (Table 3; Fig. 12). *Phaenicia caeruleiviridis* was the most common species, being observed in each of the four experimental periods (Fig. 12). The presence of other fly species varied by season. Due to this seasonal variation, the observation of carrion flies or flesh flies will be discussed by experimental period.

Experimental Period I

Two carrion fly species were found both in the field and forest sites during this initial experimental period (24 Mar-3 Apr) (Table 1). Adults of both *P. caeruleiviridis* and *C. cadaverina* were first observed on Day 4 at both the sunlit and shaded experimental plots (Fig. 13). *Cynomyopsis cadaverina* eggs were observed on the carrion the subsequent day (Day 5) at both plots; however, there was no evidence that *P. caeruleiviridis* oviposited in these cold weather conditions (Table 2; Fig. 13). First stage *C. cadaverina* larvae emerged from these eggs three days after oviposition (Fig. 13). Larval development continued into second stage over the remainder of this experimental period. Larval development was slow in these cold temperatures as evidenced by the fact that larvae never developed past this second stage into the third stage (Fig. 13).

Experimental Period II

Three fly species, *P. caeruleiviridis*, *C. macellaria*, and *S. bullata*, were collected from the carcasses during this experimental period (19 May-29 May) (Table 1; Table 3; Fig. 14). There were no colonization differences noted between male and female carrion. In these warmer temperatures (Table 2), *P. caeruleiviridis* adults were observed immediately (i.e., within 1 hour) after rat placement in both the field and forest plots (Fig. 14). Furthermore, *P. caeruleiviridis* eggs were observed on both Day 0 (sunlit and shaded) carcasses. Thus, oviposition by *P. caeruleiviridis* occurred within 2 hours when flies were exposed to these warmer conditions (Fig. 14). Larval development, too, was accelerated under these weather conditions with first and second stage larvae being found on the carrion as early as Day 1 and Day 2, respectively. By Day 3, third stage larvae of *P. caeruleiviridis* (Fig. 9 A-C), *C. macellaria* (Fig. 10 A-D), and *S. bullata* (Fig. 11 A-C) were observed on carcasses in the sunlit field plot. *Phaenicia caeruleiviridis* and *S. bullata* third stage larvae were found in the forest plot by Day 3 and Day 5, respectively (Fig. 14). *Cochliomyia macellaria* larvae were never collected from carrion in the shaded forest plot.

Experimental Period III

Four fly species, *P. caeruleiviridis*, *C. macellaria*, *S. bullata*, and *S. haemorrhoidalis*, were recorded for this experimental period (12 Jul-22

Jul) (Table 1; Table 3, Fig. 15). As was observed in experimental period II, *P. caeruleiviridis* adults were immediately attracted to both sunlit and shaded carrion (i.e., within 1 hour of rat placement in the field) and oviposition occurred within 2 hours (Fig. 15). First stage *P. caeruleiviridis* larvae developed on both sunlit and shaded carrion by Day 1 and into third stage by Day 2 (Fig. 15).

As was the case with *P. caeruleiviridis*, adults of both *S. bullata* and *S. haemorrhoidalis* were observed on Day 0 at both field and forest plots. However, of the two flesh fly species, only *S. bullata* larvae were collected during this experimental period and those were found exclusively on carcasses in sunlit conditions (Fig. 15). *Cochliomyia macellaria* adults were never observed; although they must have oviposited on the sunlit carcasses as third stage larvae were found as early as Day 3 (Fig. 15). As was noted in experimental period II, *C. macellaria* larvae were never collected from carrion in shaded conditions (Fig. 15).

Experimental Period IV

Adults of four fly species, *P. caeruleiviridis*, *C. cadaverina*, *S. haemorrhoidalis*, and *M. domestica*, were observed during this period (13 Oct-23 Oct); yet only *P. caeruleiviridis* and *C. cadaverina* larvae were collected (Table 1; Fig 16). *Phaenicia caeruleiviridis*, *S. haemorrhoidalis*, and *M. domestica* adults were observed on Day 0 while *C. cadaverina* adults were not observed until Day 1 (Fig. 16). Oviposition was delayed in

the colder temperatures as no eggs were observed until Day 2, 48 hours after rat placement in the field (Fig. 16). Eggs found were either *P. caeruleiviridis* or *C. cadaverina*, but there is no way to distinguish between eggs of the two species. Third stage *P. caeruleiviridis* larvae developed by Day 4 and Day 5 in the field and forest plots, respectively (Fig. 16). Third stage *C. cadaverina* larvae were found as early as Day 5 on carcasses in both sunlit and shaded plots (Fig. 16).

INSECTA (COLEOPTERANS)

Representatives from 10 beetle families were observed throughout the four experimental periods. Families represented were the Carabidae, Dermestidae, Histeridae, Leiodidae, Meloidae, Nitudulidae, Scarabidae, Silphidae, Staphylinidae, and Trogidae (Table 3).

While *Aphodius lividis* (Oliver), *Calathus opaculus* LeConte, and *Trox spinulosis* (Robinson) were collected only from sunlit plots, all other beetle species were more frequently associated with shaded conditions. The question, then, was; are beetles significantly predisposed to shaded conditions, or is their higher frequency of occurrence in such conditions merely a reflection of chance? To address this question, I decided to simply count all the days that a particular species of beetle was found in both sunlit and shaded conditions by each experimental period and for all experimental periods combined. For example, the hairy rove beetle, *Creophilus maxillosus* (Linnaeus), was collected on one day from sunlit

plots and five days from shaded plots during experimental period II (Fig. 17). Thus, by adding the 1 and 5 collection days for sunlit and shaded plots, respectively, one arrives at a total beetle day (TBD) figure of 6 for this particular species in experimental period II. By treating all beetle species collected in experimental period II in a similar manner, a TBD figure can be summed for all beetle species in this experimental period. Table 5 shows that the TBD (all species combined) for experimental period II was 43, with 27.9% and 72.1% of those days representing beetles collected in sunlit and shaded conditions, respectively.

The null hypothesis is that percent occurrence of beetles in sunlit conditions of experimental period II is the same as percent occurrence in shaded conditions for that experimental period. A Chi square value was calculated to test H_o. In this instance, H_o was rejected ($X^2 = 8.395$; 1 df; $P = 0.0043$); that is, beetles collected in experimental period II were significantly more likely to be found on carcasses in shaded rather than sunlit conditions (Table 5).

Throughout experimental period III, beetle collections totaled 72 TBD. Beetles were observed in the sunlit field plot a total of 14 days (19.4%) while collections totaled 58 days (80.6%) from the forest plot (Table 5). Thus, the null hypothesis that beetle occurrence is the same on sunlit versus shaded carrion in this experimental period was rejected ($X^2 = 26.889$; 1 df; $P > 0.0001$) (Table 5).

The TBD for experimental period IV was 35, 25.7% of which were collections from carcasses exposed to sunlit conditions and 74.3% from carcasses in shaded conditions (Table 5). As was the case with all other experimental periods examined, the null hypothesis that the percentage occurrence of beetles is the same on sunlit versus shaded carcasses during this experimental period was rejected ($X^2 = 8.257$; 1 df; $P = 0.0045$) (Table 5).

Experimental Period I

No beetle species were found associated with the carrion during this 11 day experimental period (24 Mar-3 Apr) (Table 1). However, several rat carcasses were left in the field beyond the experimental period and *Geotrupes blackburnii* (Fabricius) and *Nicrophorus orbicollis* Say were found on carcasses after the end of the experimental period (i.e., after 11 days) (Table 3).

Experimental Period II

Fifteen beetles species and one hemipteran were collected during this experimental period (19 May-29 May) (Table 2; Fig. 17). With the exception of *Necrodes surinamensis* and *Necrophila americana*, beetle species did not appear on carcasses until Day 4 of this experimental period (Fig. 17) where temperatures readings (Fig. 6) hovered around the 20 °C mark. Only six species were observed at the sunlit plot during this

experimental period including: *A. lividis*, *Euspilotus assimilis* (Paykull), *Necrophila americana* (Linnaeus), *C. maxillosus*, a small unidentified species, and *T. spinulosus* (Fig. 17). Other species were found only in shaded conditions. Only *A. lividis* was found exclusively in sunlit conditions (Fig. 17).

Experimental Period III

Sixteen beetle species were collected during this experimental period (12 Jul-22 Jul), the most common being *Chlaenius cericeus* LeConte, *E. assimilis*, and *N. americana* (Table 1; Table 3; Fig. 18). *Necrophila americana* was encountered more frequently than any other beetle species during this experimental period. It was not uncommon to find anywhere from 20 to 66 *N. americana* individuals on a given day, whereas counts of other beetles were generally less than 10 individuals per species. Higher temperatures, characteristic for this experimental period (Fig. 6), appeared to favor more rapid colonization of carcasses, as 4 beetle species were found as early as Day 1 and 12 species were present by Day 3 (Fig. 18). Only six beetle species were found associated with sunlit carcasses; *E. assimilis*, *Necrodes surinamensis* (Fabricius), *N. americana*, *N. orbicollis*, *Nicrophorus tomentosus* Weber, and *C. maxillosus* (Fig. 18).

Experimental Period IV

Fourteen beetle representatives were found during the last experimental period (13 Oct-23 Oct), 5 of which were collected from sunlit carrion (Table 1; Fig. 19). As in experimental period II, cooler temperatures (Fig. 6) seemed to delay beetle colonization of carcasses (Fig. 19). Only 5 species were collected from carcasses by Day 3, considerably fewer species than were found by Day 3 of the previous experimental period. *Calathus opaculus* and *T. spinulosus* were collected exclusively from carcasses in sunlit conditions (Fig. 19). *Creophilus maxillosus* was the most common beetle observed during this period, being collected a total of 8 days; 4 from the sunlit plot and 4 from the shaded plot (Fig. 19).

INSECTA (HEMIPTERA)

One *Georchis* sp. was collected on the shaded Day 6 carcass of experimental period II (Fig. 17). No other individuals were observed throughout the four experimental periods.

INSECTA (HYMENOPTERANS)

Unidentified ants and yellow jackets were observed associated with carrion in experimental periods III and IV (Table 3). Yellow jackets were attracted to the carrion immediately (i.e., within 1 h) after placement in the field.

CRUSTACEA (ISOPODA)

Unidentified pill bugs were found associated with carrion in experimental periods I, III, and IV (Table 3).

GASTROPODA (STYLOMMATOPHORANS)

An unidentified snail species and the slug *Deroceras reticulates* (Muller, 1774) were observed on the carcasses during experimental period IV (Table 3). Slugs were commonly observed invading body orifices (i.e., the eye orbital area, nasal openings, or anus) and the ventral abdominal incision (Fig. 20 A-C). Slugs were present only on fresh carcasses (Day 0—Day 4) and left after the carcasses lost moisture.

CHAPTER 6

DISCUSSION

A total of 43 species were observed throughout the four experimental periods, which is comparable to other successional studies conducted on small mammal carrion (Johnson, 1975; Tomberlin and Adler, 1998; DeJong and Chadwick, 1999); but fewer species than noted by other faunal succession studies. Bourel et al. (1999) collected 66 arthropod species from rabbit carrion in sand dune habitats in Northern France. Impressively, Payne (1965) recorded a total of 522 species from the baby pig, *Sus scrofa*.

MYRIAPODS (CHILOPODS AND DIPLOPODS)

Even though millipedes and centipedes are considered common inhabitants of North America (Pechenik, 2000), they are seldom seen because they are secretive, nocturnal animals that also require moist conditions because they cannot regulate body moisture like their insect relatives (i.e., myriapod spiracles remain open, whereas spiracles of insects can close retaining moisture within the body). These animals, being secretive under normal circumstances, are less likely to be observed in forensic studies. For example, Payne (1965), working on baby pigs, *Sus scrofa*, in South Carolina, found 522 species, representing 3 phyla, 9 classes, 31 orders, 151 families, and 359 genera, but mentioned no myriapod species. Other studies of arthropod succession

also reflect this myriapod absence (Reed, 1958; Johnson, 1975; Rodriquez and Bass, 1983; Richards and Goff, 1997; Tomberlin and Adler 1998; DeJong and Chadwick 1999).

Myriapod presence is not, however, entirely unheard of since Hoffman and Payne (1969) indicated reported carnivory among members of four orders of Diplopoda. Payne and Crossley (1966) discovered 10 species of millipedes from 7 orders, on or around the bodies of baby pigs at Clemson, South Carolina. Millipedes were also observed by Bourel et al. (1999), who reported *Iulus sabulosus* Linnaeus frequenting carrion. Catts and Haskell (1997) indicate that centipedes, which are predatory on other small animals, and a number of millipede species, which feed on animal tissues, are often recovered from carrion. Centipedes and millipedes are generally considered carnivores or detritivores (Pechenik, 2000), so it is likely that they are visiting carcasses to feed on small organisms or detritus. Hoffman (pers. comm., 1999) noted that none of the three myriapod species observed in this study "...have been recorded as feeding on animal tissues."

Myriapods were found only during the cooler, experimental period I (24 Mar-3 Apr '99) and were associated more frequently with carrion in shaded conditions as compared to carrion exposed in the sunlit plot (Table 4). It stands to reason that myriapods are not often encountered in warm, sunlit conditions since they cannot adequately retain body moisture.

INSECTA (DIPTERANS)

Seven species of flies were observed throughout the course of this experiment: 3 calliphorids (*Cynomyopsis cadaverina*, *Phaenicia caeruleiviridis*, and *Cochliomyia macellaria*), 2 sarcophagids (*Sarcophaga bullata* and *S. haemorrhoidalis*), and 2 muscids (*Musca domestica* and *Fannia canicularis*). Since the muscid flies were seldom encountered and are not generally considered carrion feeders, there will be no further discussion of these flies. For convenience, each of the remaining fly species will be discussed separately.

Phaenicia caeruleiviridis

The most frequently observed calliphorid was the green-bottle fly, *Phaenicia caeruleiviridis*, which associated with both sunlight and shaded carrion in each of the four experimental periods. Reed (1958), working with dog carcasses in Tennessee, also noted that *P. caeruleiviridis* was the predominant fly. Hall and Doisy (1993) studying blowfly attraction and oviposition in chicken carrion in Missouri, similarily observed abundant populations of *P. caeruleiviridis*.

Adults of *P. caeruleiviridis* were first recorded on Day 4 of experimental period I (Fig. 13) landing on carrion in both the sunlit and shaded plots. Although temperatures were high enough to allow adult activity, environmental conditions must have not have been favorable for oviposition by *P. caeruleiviridis* females since no *P. caeruleiviridis* larvae

were collected from the carrion (Fig. 13). This corroborates the findings of Bourel et al. (1999), who demonstrated that even if daytime temperatures are apparently favorable for adult blowfly activity, oviposition may be delayed by low nighttime temperatures. It has also been demonstrated that the temperature of the carcass must reach a required threshold before some species (i.e., *Phaenicia sericata*) will oviposit (Keh, 1985). Or perhaps environmental conditions did allow for *P. caeruleiviridis* oviposition, but were unfavorable for any further development of the observed eggs.

Unlike experimental period I, where cooler temperatures delayed adult fly arrival, *P. caeruleiviridis* adults were immediately attracted to carrion exposed in the warmer experimental periods II and III (Table 2; Figs. 14 and 15). Oviposition occurred within 2 hours after rat placement in both the field and forest plots in these warmer conditions (Fig. 14). Similarly, Hall and Doisy (1993) commonly observed *P. caeruleiviridis* 24 - 48 hours after death of the chicken host. *Phaenicia caeruleiviridis* is a noted early arriver and predominates in adult trap catches over fresh carrion throughout the mid-United States (Hall, 1948; Hall and Townsend, 1977; Hall, 1979). Additionally, the warmer temperatures in experimental period II and III (Table 2) allowed for rapid larval development. Third stage *P. caeruleiviridis* larvae were collected as early as Day 3 in experimental period II and Day 2 in experimental period III (Figs. 14 and 15).

As was observed in experimental periods II and III, adult *P. caeruleiviridis* flies were immediately attracted to both the carrion placed in sunlit and shaded conditions, but as was noted in experimental period I, *P. caeruleiviridis* oviposition was delayed by the cooler temperatures in experimental period IV (Table 2; Fig. 16). Eggs were not observed in experimental period IV, however, on the carrion until 48 hours after rat placement in the field (Fig. 16). Thus, environmental conditions must not have been favorable for oviposition by *P. caeruleiviridis* females. Once oviposition occurred, larval development was rapid as third stage *P. caeruleiviridis* larvae were collected from carrion by Day 4 and 5 in the sunlit and shaded plots, respectively (Fig. 16).

Like other studies have demonstrated, *P. caeruleiviridis* was observed to be an early arriver on freshly exposed carrion, but oviposition was dictated by environmental conditions (Hall, 1948; Hall and Townsend, 1977; Hall, 1979; and Hall and Doisy, 1993).

Cochliomyia macellaria

Unlike Tomberlin and Adler (1998) who recorded *Cochliomyia macellaria* as being the most abundant insect colonizing rat carrion in their study, this species, distinct for its double-tipped spines on larval segments (Fig. 10 D), was not nearly as common as *Phaenicia caeruleiviridis* in the present study. Likewise, in a study conducted on chicken carrion by Hall

and Doisy (1993), adult *C. macellaria* flies accounted for only ≈1% of all flies trapped.

Cochliomyia macellaria larvae were observed only in the two warmer experimental periods (II and III) (Fig. 12). Additionally, larvae were only found on carrion exposed in the sunlit field plot (Fig. 12), thus further indicating that perhaps *C. macellaria* prefers warmer temperatures. In fact, Byrd and Butler (1996) demonstrated that the preferred mean development temperature of *C. macellaria* larvae after 24 hours is 35 ± 0.8 °C.

Cynomyopsis cadaverina

Cynomyopsis cadaverina, distinctive for the presence of an accessory oral sclerite in larval stages (Fig. 8 B), is known as a cold weather species (Hall and Doisy, 1993). Not surprisingly, then, *C. cadaverina* was observed only in experimental periods I and IV when temperatures were cooler (Fig. 12). Thus, the observation of *C. cadaverina* only in experimental periods I and IV when temperatures were cooler, corroborates the findings of Hall and Doisy (1993).

The female blowfly must oviposit at a wound site or natural orifice since newly hatched larvae are generally incapable of penetrating unbroken integument (Byrd, 1995). *Cynomyopsis cadaverina* eggs were most commonly observed around the nose, in the mouth, and around the anus even though an incision had been made in the abdomen for easy

access. Oviposition site preferences vary between species, and Greenberg (1991) noted that the preferred sites of *C. cadaverina* are (in order) mouth, nose, anus, and wound site and that this preference does not change between carrion exposed to sunlit versus shaded conditions.

***Sarcophaga* spp.**

Two species of sarcophagid flies were observed throughout the four experimental periods; *Sarcophaga bullata* and *S. haemorrhoidalis*. *Sarcophaga* spp. adults were recorded in every experimental period, except experimental period I, but sarcophagid larvae were only collected from carrion in the warmer experimental periods (II and III) (Fig. 12). Similarly, other studies have observed *Sarcophaga* spp. in summer months (Payne, 1965; Tomberlin and Adler, 1998; Bourel et al., 1999; DeJong and Chadwick, 1999). Deonier (1940) noted that the minimum temperature range for adult *Sarcophaga* spp. activity is 50 – 60 °C. In the present study, *Sarcophaga bullata* was found in experimental periods II and III, whereas *S. haemorrhoidalis* adult individuals were observed in experimental periods III and IV (Fig. 12).

Hall (1948) noted that "*Sarcophaga* species are commonly attracted to baits which are very old and strong." Reed (1958) and Rodriguez and Bass (1983) also observed *Sarcophaga* spp. as being one of the later-arriving taxa. Conversely, Hall and Doisy (1993) demonstrated that some sarcophagids are among the first arrivals at carrion. The

present study supports this latter finding in that *S. bullata* and *S. haemorrhoidalis* adults were immediately sighted around carrion in experimental period II (Fig. 15). Additionally, *S. haemorrhoidalis* adults were immediately associated with the carrion in experimental period IV (Fig. 16).

Sarcophagid larval populations were noticeably less abundant than their calliphorid competitors. This is possibly due, in part, to the fact that calliphorid flies deposit many more eggs (≈250) than can sarcophagid females lay larvae. Knipling (1936) demonstrated that *Sarcophaga* sp. produced the smallest number of larvae, the average of five females being 6.4 larvae.

Sarcophaga spp. larvae are visibly different from calliphorid larvae. Sarcophagid larvae are distinctly larger than blowfly larvae and their larval spiracles also differ from those of their calliphorid relatives. The posterior spiracles of sarcophagid larvae are situated in a depressed area located on the last segment (Fig. 11 A and B).

INSECTA (COLEOPTERANS)

Carabid, dermestid, histerid, leiodid, meloid, nitudulid, scarabid, staphylinid, and trogid beetles were collected in the present study, which is comparable to the 10 beetle families found by Bourel et al. (1999). Additional studies, too, have observed multiple coleopteran families frequenting carrion (Reed, 1958; Payne, 1965; Johnson, 1975; Rodriguez

and Bass, 1983; Braack, 1987; Richards and Goff, 1977; DeJong and Chadwick, 1999).

Histerids are primarily predators and their preferred food source is probably maggots (Johnson, 1975). Other fly larvae predators include staphylinids and silphids. In fact, Payne (1965) observed histerids and staphylinids eating fly larvae. Thus, these predatory beetles must arrive at the carcass early on when larvae are present as was generally observed in the present study (Figs 17, 18, 19) as well as in past studies (Rodriguez and Bass,1983; Bourel et al., 1999). Then too, temperature appeared to be a contributing factor in the speed of colonization of carcasses by beetles. The most rapid colonization occurred in July (experimental period III) when temperatures averaged nearly 30 °C for that 11 day period. And there were no beetles present during the coolest experimental period of March/April.

Beetles were significantly more likely to be found associated with carrion in the shaded forest plot as compared to carrion in the sunlit field site ($X^2 = 42.667$; $P > 0.0001$). This supports the observations of Reed (1958) who noted that carcasses in wooded areas had larger insect populations than carcasses in pasture areas. Bourel et al. (1999) also observed that the greatest diversity of arthropods came from carcasses located in a wooded site.

MISCELLANEOUS TAXA

Ants, yellow jackets, pill bugs, and snails were not identified, nor were their developmental patterns examined. It should be noted, however, that ants, yellow jackets, and pill bugs have been reported in numerous arthropod successional studies (Payne, 1965; Johnson, 1975; Richards and Goff, 1997; and Bourel et al., 1999). The slug *Deroceras reticulatus* was commonly observed invading various body openings in the early days of experimental period IV. There are no known records in the literature documenting such activity on the part of these mollusks. Still, the observed slug invasions were so aggressive, and of such duration, that they cannot be dismissed as casual or accidental encounters. By Day 4, all slugs had left the desiccating carrion.

CHAPTER 7

SUMMARY

Previous studies have demonstrated a general trend during carrion decomposition in that Diptera, which initially invade the carcass, are replaced by Coleoptera acting as predators on the maggots (Reed, 1958; Payne, 1965; Johnson, 1975; Rodriguez and Bass, 1983; Braack, 1987; Richards and Goff, 1977; Bourel et al., 1999; DeJong and Chadwick, 1999). The current study was no exception in demonstrating this general trend. The dominant insect orders found associated with carrion in this study conducted in West Virginia were Diptera and Coleoptera.

Seasonal variation of arthropod succession on carrion did occur in that *Cochiomyia macellaria* and *Sarcophaga* spp. larvae were only encountered during the warmer experimental periods (II and III), whereas *Cynomyopsis cadaverina* was only observed in the colder experimental periods (I and IV). Temperature also had an effect on beetle colonization of the carrion in this study in that the most rapid colonization occurred in July (experimental period III) and there were no beetles recorded from the coolest experimental period of March/April. There were no colonizational differences between male versus female carcasses.

The current study corroborates the findings of Reed (1958) and Bourel et al. (1999) who noted that the greatest diversity of arthropods came from carrion in a wooded site as compared to carrion in pasture

areas. Beetles were significantly more likely to be associated with carrion in the shaded forest plot in this study.

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Table 1. Starting and ending dates (1999) for the four experimental periods at Green Bottom Wildlife Management Area.

Experimental Period	Day 0	Day 10
I	24 Mar	3 Apr
II	19 May	29 May
III	12 Jul	22 Jul
IV	13 Oct	23 Oct

Table 2. Climatological data for sunlit and shaded plots at GBWMA field site for the four experimental periods shown in Table 1. All temperatures are given in °C; relative humidity in %; and rainfall in mm. NA = not available.

		Sunlit											Shaded										
	DAY	0	1	2	3	4	5	6	7	8	9	10	0	1	2	3	4	5	6	7	8	9	10
Experimental Period I Day 0=24 Mar	Ambient	7.5	7	6	9	12	11	14	16	13	NA	NA	7.5	7	6	9	9	14	12	16	11.5	13	18.5
	Ground surface	8.5	10	7	7.5	16	17	11	12	13	NA	NA	8.5	10	7	7	7	16	9	15	12	12.5	16
	Soil (10 cm)	6	6	6.5	6	9	9	8	8	10	NA	NA	6	6	6.5	4.5	6	9	8	8	10	11	13
	Body/ground	NA	5	6	6.5	12.5	10	7	12	12	NA	NA	NA	5	5.5	8.5	7	11	7	8.5	12	12	11.5
	RH (%)	78	54	71	66	58	54	46	48	85	NA	NA	78	54	71	66	58	52	46	55	88	77	72
	Rainfall (mm)	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	6	0	0
Experimental Period II Day 0=19 May	Ambient	22	21	24	27	20.5	17	20	22	22	23	21	22	19	21.5	22	19	14	17	21	19	18.5	17
	Ground surface	21	30	33	28	23	21.5	22	31	21	23	18	20	18	21.5	22	19	14	17	20	19	21	17.5
	Soil (10 cm)	20	18.5	19	20	19.5	17	23	17	19	22	17	18	16	16.5	17	17	14.5	15	17	15	17	15.5
	Body/ground	NA	27	28	26	NA	NA	NA	NA	NA	NA	NA	NA	19	18.5	21.5	NA	NA	NA	NA	NA	NA	NA
	RH (%)	69	51	57	78	90	74	22	58	62	60	76	62	66	62	72	90	68	51	62	51	60	54
	Rainfall (mm)	0	0	0	0	2	4	0	0	0	0	0	0	0	0	0	2	4	0	0	0	0	0
Experimental Period III Day 0=12 Jul	Ambient	29.5	25	29	31.5	86	33	30.5	34	25	30.5	31	26	25	28	30	29	29	29	31	25	29	30
	Ground surface	41.5	42.5	35	42	42	44	38	43	29	33	39	24.5	23	27	33	30	33	30	35	27	29	29
	Soil (10 cm)	29	23	25	25	27	29	26	29	27	25	26	21	19	22	22	22	25	22	23	24	23	24
	Body/ground	29	25	39	41	31	37	32	40	28	34	37	23	22	36	36	26	29	28	30	26	27	32
	RH (%)	48	71	61	50	52	46	64	44	92	66	74	60	60	59	52	56	66	70	64	92	74	76
	Rainfall (mm)	0	0	0	0	0	0	3	0	6	0	4	0	0	0	0	0	0	3	0	6	0	4
Experimetnal Period IV Day 0=13 Oct	Ambient	23	12	14	18	14.5	12	14	12	11	15	7.5	22	13	14	17	15	11	13	12	11	14.5	7.5
	Ground surface	25	22	15	22	18	18	19.5	20	12	14	11	22	14	13	17	16	13	14	12	12	13	10
	Soil (10 cm)	17	15	13.5	15	15	15	13.5	13	14	12.5	12	17	15	13	15	14	14	13	12	12	12	12
	Body/ground	24	14	16	18	16	17	18.5	14	15	17	12	23	14	14	16	16	14	21.5	15	12.5	NA	11
	RH (%)	66	66	68	62	86	72	67	76	52	54	66	68	52	68	70	88	70	81	70	62	46	66
	Rainfall (mm)	0	12	0	0	0	0	0	1	0	0	0	0	12	0	0	0	0	0	1	0	0	0

Table 3. All taxa observed at GBWMA throughout the four experimental periods in 1999. Roman numerals indicate Experimental Period (see Table 1 for starting and ending dates).

	I	II	III	IV
PHYLUM ARTHROPODA				
Class Chilopoda				
Cryptopidae:				
Scolopocryptops sexspinosus (Say)	•			
Class Diplopoda				
Order Julida				
Julidae:				
Ophyiulus pilosus (Newport)	•			
Polydesmidae:				
Pseudopolydesmus sp. ?	•			
Class Insecta				
Order Coleoptera				
Carabidae:				
Aphodius lividus (Oliver, 1789)		•		
Calathus opaculus LeConte, 1854				•
Chlaenius cericeus ? (Forster)		•	•	•
Pterostichus permundus (Say)				•
small unidentified sp.				•
Dermestidae:				
Dermestes sp. Linnaeus				•
Histeridae:				
Euspilotus assimilis (Paykull, 1811)		•	•	
Hister abbreviatus Fabricius, 1775				•
Leiodidae:				
unidentified sp.				•
Meloidae:				
Meloe impressa ? Kirby 1837		•		•
Nitudulidae:				
Omosita colon (Linnaeus, 1758)		•	•	
Scarabidae:				
Geotrupes blackburnii (Fabricius, 1781)	•			
Onthophagus hecate (Panzer, 1794)		•		
Onthophagus orpheus (Panzer, 1794)			•	
Silphidae:				
Necrodes surinamensis (Fabricius, 1775)		•	•	
Necrophila americana (Linnaeus, 1759)		•	•	
Nicrophorus orbicollis Say, 1825	•	•	•	

Table 3 continued

	I	II	III	IV
Nicrophorus tomentosus Weber, 1801			•	•
Oiceoptoma inaequalis (Fabricius, 1781)		•	•	
Oiceoptoma noveboracense (Forster, 1771)		•	•	
Staphylinidae:				
Creophilus maxillosus (Linnaeus, 1758)		•	•	•
Ontholestes cingulatus (Gravenhorst, 1802)			•	•
Platydracus maculosus (Gravenhorst, 1802)			•	
Philonthus cyanipennis (Fabricius, 1792)				•
small unidentified sp.		•	•	•
Trogidae:				
Trox spinulosus (Robinson, 1940)		•	•	•
Trox unistriatus (Beauvois, 1805)		•	•	
Order Diptera				
Calliphoridae/Calliphorinae:				
Cynomyopsis cadaverina (Robineau-Desvoidy)	•			•
Phaenicia caeruleiviridis (Macquart)	•	•	•	•
Calliphoridae/Cochliomyinae				
Cochliomyia macellaria (Fabricius)		•	•	
Muscidae:				
Musca domestica Linnaeus				•
Fannia canicularis (Linnaeus) larva			•	
Sarcophagidae:				
Sarcophaga bullata Parker		•	•	
Sarcophaga haemorrhoidalis (Fallen)			•	•
Order Hemiptera:				
Lygaedidae:				
Geochoris sp.		•		
Order Hymenoptera				
unidentified ants			•	•
unidentified yellow jackets			•	•
Class Crustacea				
Order Isopoda				
Armadillidiidae:				
unidentified "pill bug"	•		•	•
PHYLUM MOLLUSCA				
Class Gastropoda				
Order Stylommatophora				
unidentified snail				•
Agriolimacidae				
Deroceras reticulates (Muller, 1774)				•

Table 4. Numbers of myriapod (chilopod and diplopod) individuals observed associated with sunlit and shaded carrion throughout experimental period I (24 Mar-3 Apr '99).

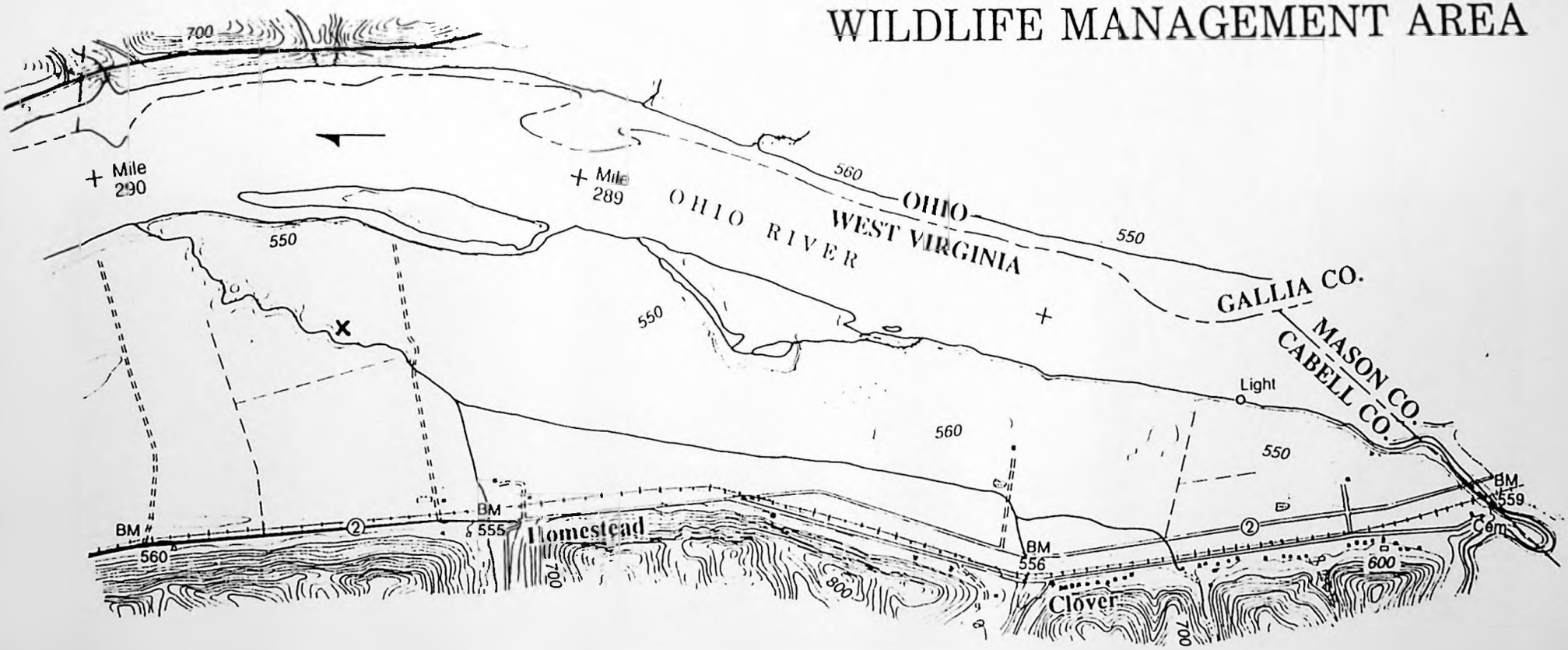
DAY	*Ophyiulus pilosus*		*Pseudopolydesmus* sp.		*Scolopocryptops sexspinosus*	
	Sunlit	Shaded	Sunlit	Shaded	Sunlit	Shaded
0	0	0	0	0	0	0
1	1	0	0	0	0	0
2	0	3	0	0	0	0
3	0	4	0	0	0	0
4	0	3	0	0	0	0
5	0	24	1	1	0	0
6	4	11	0	0	0	0
7	0	11	0	0	0	0
8	0	54	0	0	0	0
9	0	55	0	0	0	0
10	0	55	0	2	0	1
TOTALS	5	220	1	3	0	1

Table 5. Total days when beetles were present (TBD) in sunlit versus shaded conditions by experimental period. Occurrence data by sunlit and shaded plot conditions were compiled from Figs. 13, 14, and 15.

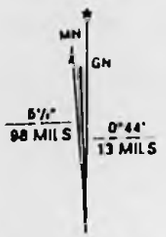
Experimental Period	Total Beetle Days (TBD)	Beetle Days Sunlit Plot	% TBD Sunlit	Beetle Days Shaded Plot	% TBD Shaded	Sun vs Shade X^2	Sun vs Shade *P*
I	0	0	0	0	0	--------	---------
II	43	12	27.9	31	72.1	8.395	0.0043
III	72	14	19.4	58	80.6	26.889	> 0.0001
IV	35	9	25.7	26	74.3	8.257	0.0045
All	150	35	23.3	115	76.7	42.667	> 0.0001

Figure 1. Map of Green Bottom Wildlife Management Area. General field site location indicated by "X".

GREEN BOTTOM
WILDLIFE MANAGEMENT AREA



ATHALIA, OHIO - W. VA. AND GLENWOOD, W. VA. - OHIO



UTM Grid and 1990 Magnetic North

SCALE 1:24 000

1 0 1 MILE

1000 0 1000 2000 3000 4000 5000 6000 7000 FEET

1 5 0 1 KILOMETER

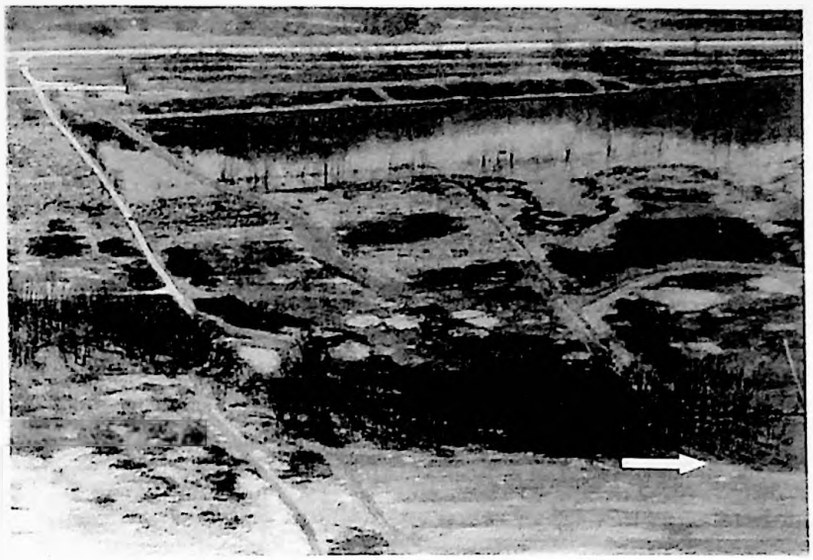
Contour Interval 20 Feet
Dotted Lines Represent 10-Foot Contours
National Geodetic Vertical Datum of 1929

Figure 2. A. Aerial view of GBWMA experimental site in March 1999 when trees were devoid of leaves. Arrow indicates general location of sunlit and shaded plots. WV Rt. 2 runs across top of photo.

B. Ground level of field (sunlit) plot, Experimental Period I (March). Note that trees in background are devoid of leaves.

C. Ground level view of forest (shaded) plot, Experimental Period I, when trees were devoid of leaves.

A



B

C

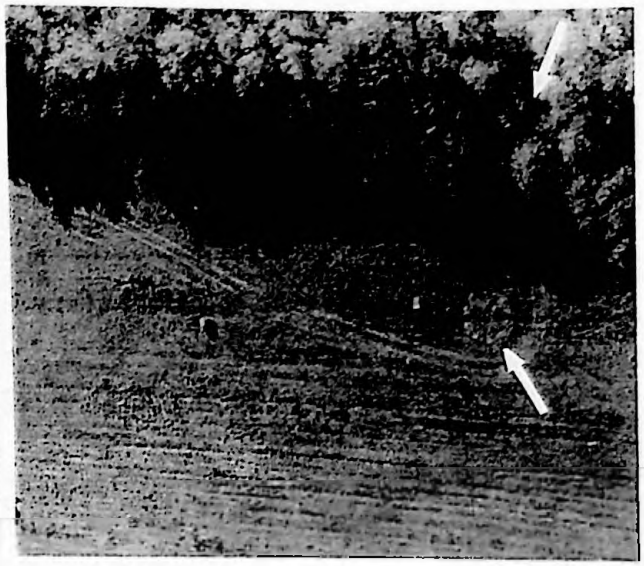
Figure 3. A. Aerial view of GBWMA experimental site taken in October 1999 when trees were in full leaf canopy. This was typical for Experimental Periods II, III, and IV. Arrow indicates general locations of sunlit and shaded plots. WV Rt. 2 runs across the bottom of photo with Ohio River seen across top of photo. WVDNR field office can be seen in lower right.

B. Aerial view of GBWMA directly over (approximately 1,000 feet) experimental plots. Lower arrow indicates sunlit plot, upper arrow indicates general location of shaded plot.

C. Ground level view of sunlit plot. Note the trees in background are in full leaf canopy.

D. Ground level view of shaded plot with trees in full canopy. Brent Weaver (left) and Dwayne Lewis (right).

A



B

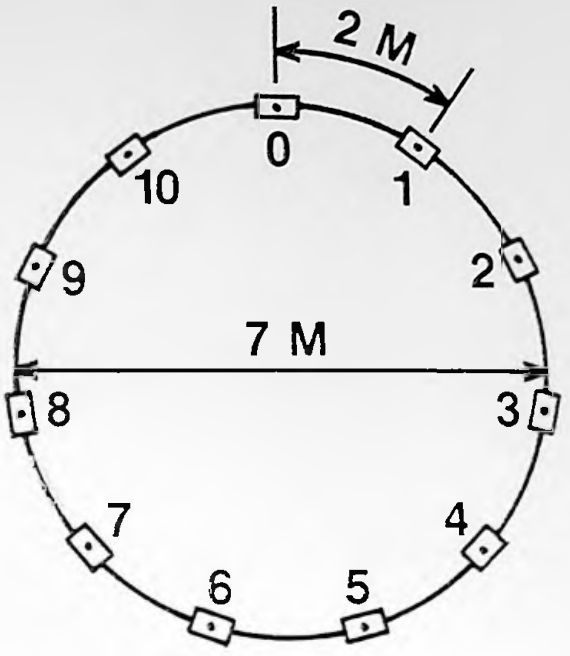
C

D

Figure 4. A. Plot design for rat placement for each experimental period (one such plot in sunlit conditions, one in shaded). Rectangular boxes represent plastic containers (50 cm long X 30 cm wide X 15 cm deep) placed in depressions dug so that upper rim of container was flush with ground surface. The center point of each box represents rat placement. Numbers 0 through 10 represent rat collection days.

B. Ground level photo of sunlit plot (March, Experimental Period I-Day 0). "Chicken-wire" metal hexnetting (2.5 cm mesh) was secured to rectangular wood frames which were placed over top of plastic containers to protect rat carcasses from scavengers/predators.

A

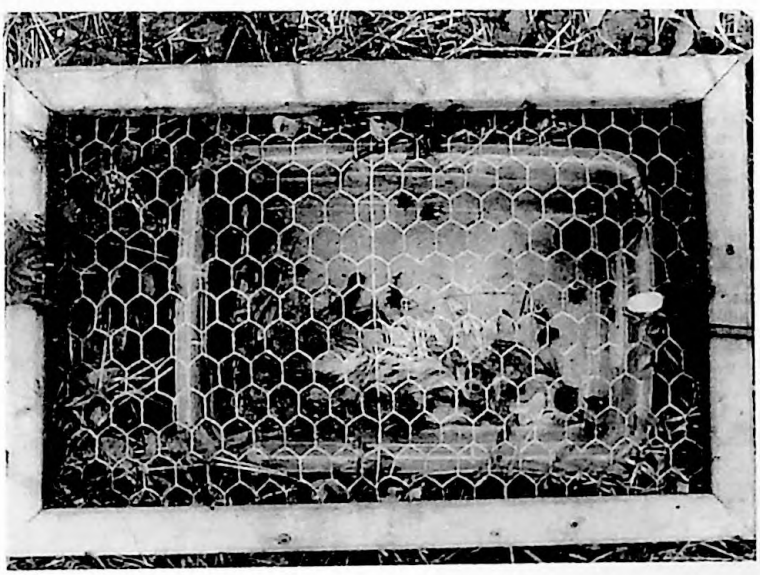


B

Figure 5. A. Example of rat placement within depression.

B. Example of container secured with framed hexnetting held in place by metal stakes.

A



B

Figure 6. Ambient temperature data for Experimental Periods (EP) I through IV (A—D). Open circles and closed circles represent on-site (GBWMA) temperatures measured daily between 11:30 AM and noon in sunlit and shaded areas, respectively. Vertical lines through measured temperature plots indicate temperature ranges for each experimental day as recorded at Lesage, West Virginia (National Weather Service Climatological Observations, Charleston, WV, 1999).

Figure 6

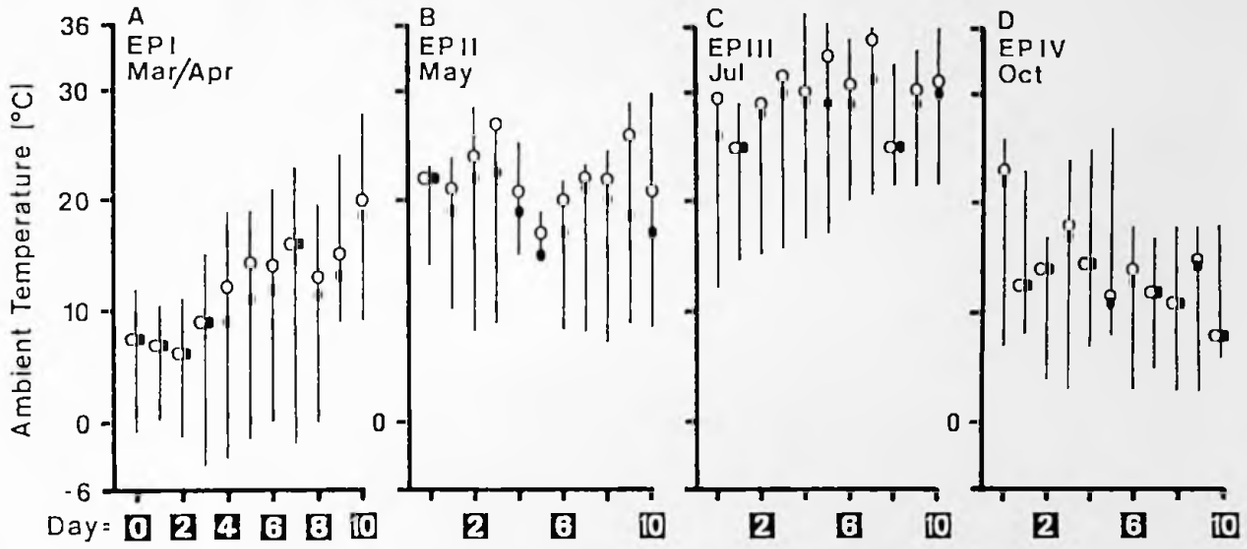

A
EP I
Mar/Apr
B
EP II
May
C
EP III
Jul
D
EP IV
Oct
Ambient Temperature [°C]
36
30
20
10
0
-6
Day=
0
2
4
6
8
10

Figure 7 A-B. Degradation of rat carcasses over time: A—1 day; B—3 days. Decay rates varied with season and weather conditions.

A

B

Figure 7 C-E. Degradation of rat carcasses over time: C--3 days; D and E—8 days. Decay rates varied with season and weather conditions.

C

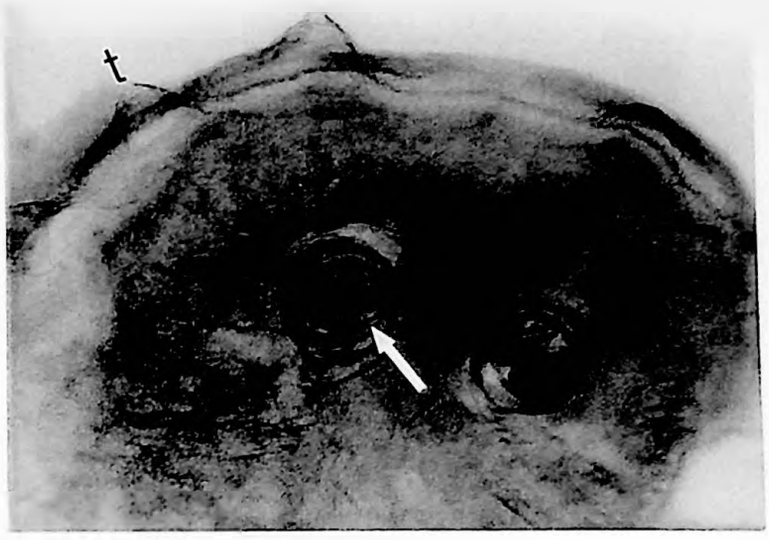
D

E

Figure 8. A. Posterior spiracles of *Cynomyopsis cadaverina* third stage larva. Note the complete peritreme and well-defined button (arrow) and tubercles (t).

B. Cephaloskeleton of *C. cadaverina* third stage larva. Note the presence of an accessory oral sclerite (arrow).

A



B

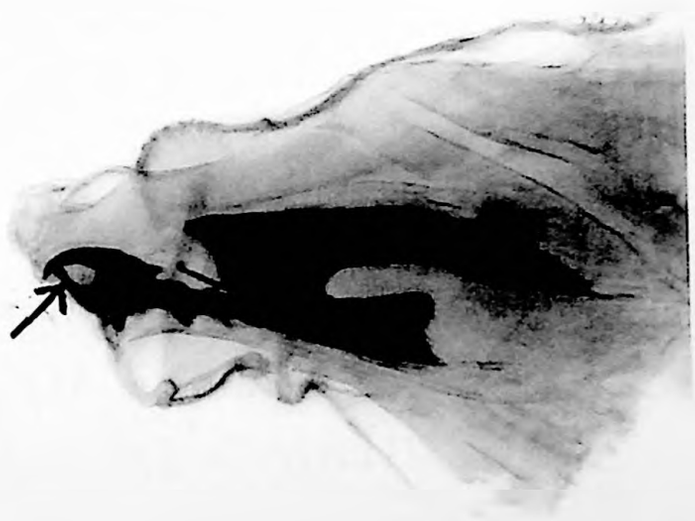
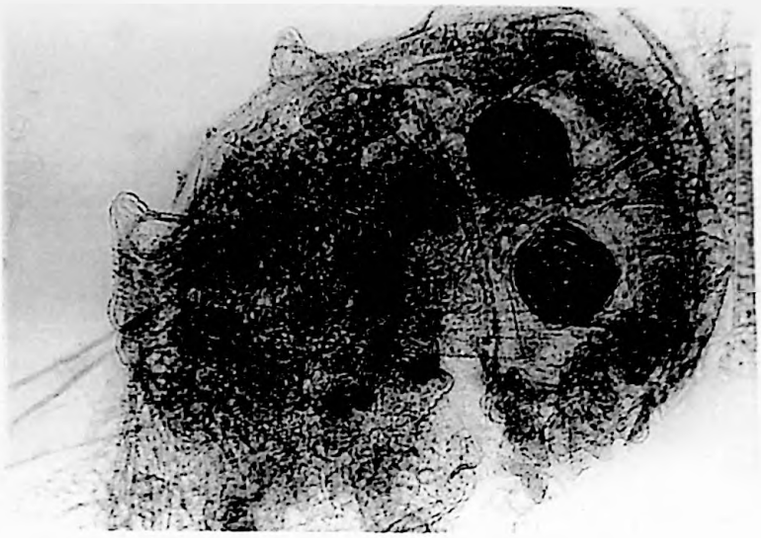


Figure 9. A. Whole view of the most posterior segment *of Phaenicia caeruleiviridis* third stage larva.

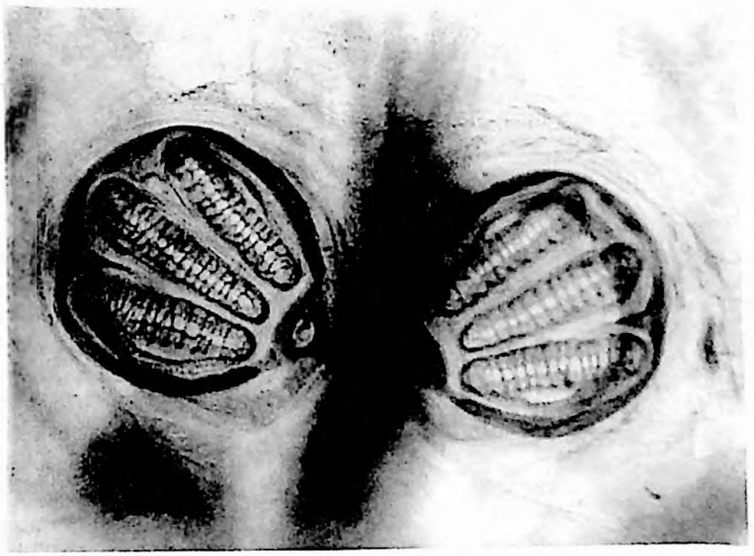
B. Close-up view of third stage *P. caeruleiviridis* spiracles. Note the complete, well-sclerotized peritreme with sharp, inward projections and the presence of a well-defined button.

C. Cephaloskeleton of *P. caeruleiviridis* third stage larva. Note the absence of an accessory oral sclerite.

A



B



C

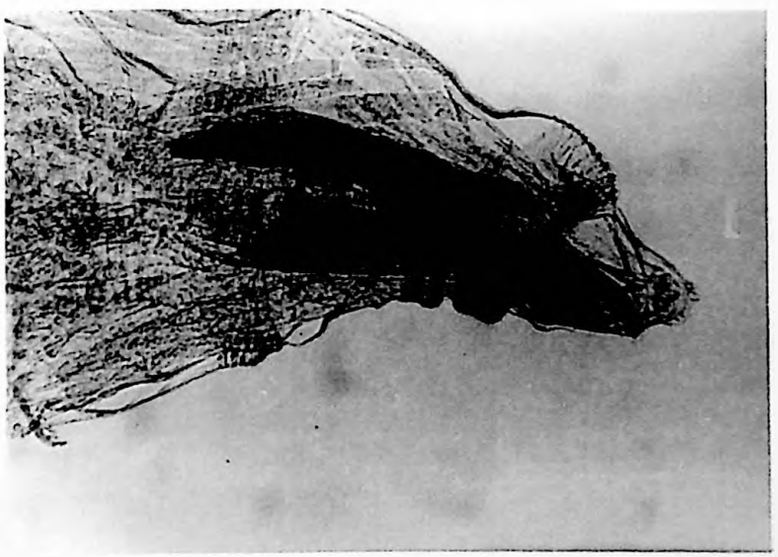
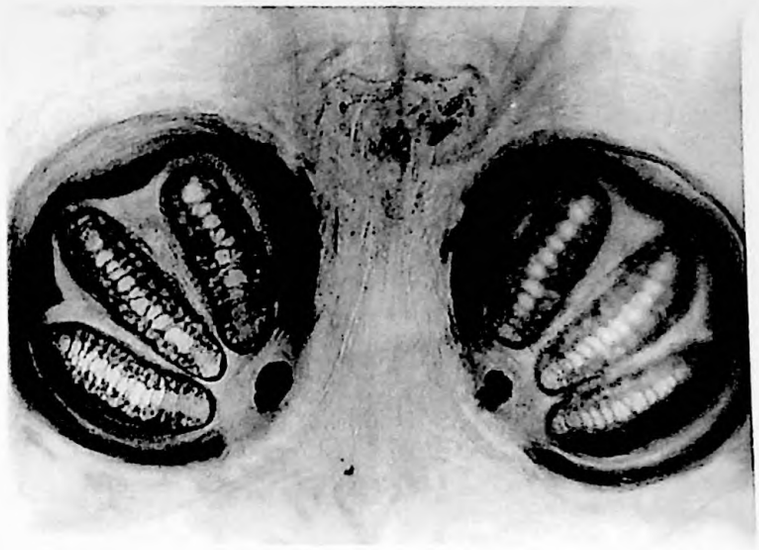


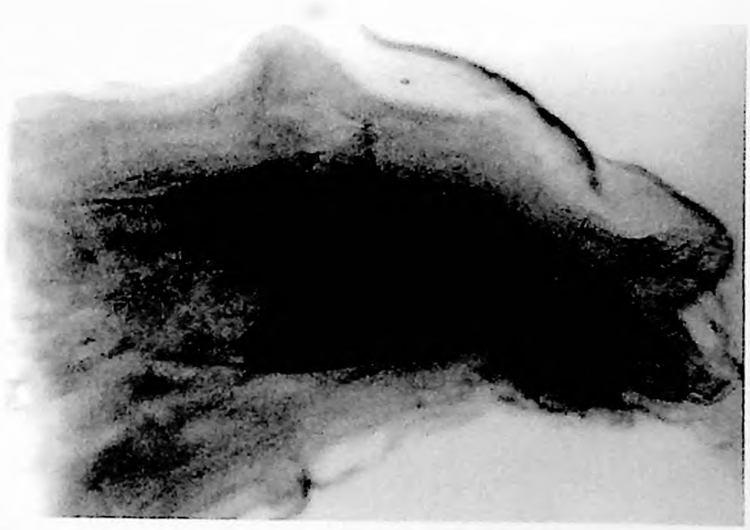
Figure 10. A. Posterior spiracles of *Cochliomyia macellaria*. Note the thick, incomplete peritreme with dull, inward projections.

B. Cephaloskeleton of *C. macellaria* third stage larva.

A



B



C. Anterior spiracles located on the anterior segment of *C. macellaria* third stage larva.

D. Note distinctly sclerotized double-tipped spines of *C. macellaria* third stage larva.

C

D

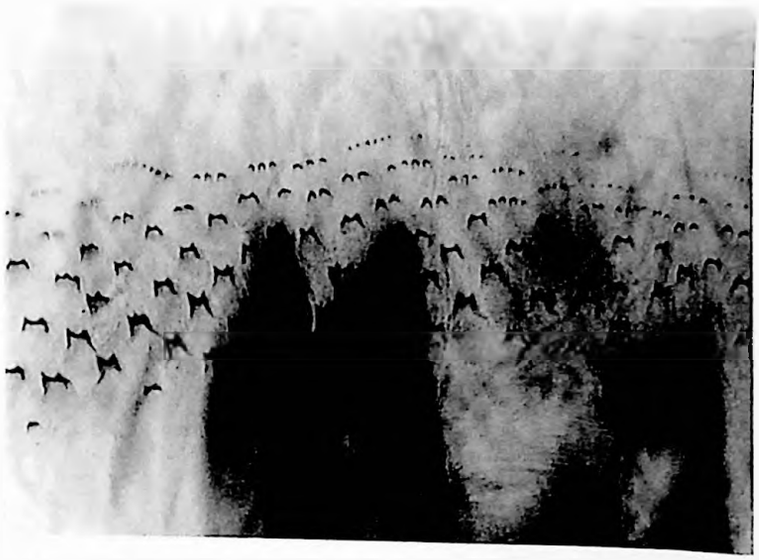


Figure 11. A. Outer margin of pit located on the most posterior segment of *Sarcophaga bullata* third stage larva. Note that spiracles are located at the bottom of this pit.

B. Third stage *S. bullata* posterior spiracles located within a pit. Note the presence of an incomplete peritreme.

C. Cephaloskeleton of *S. bullata* third stage larva.

A

B

C

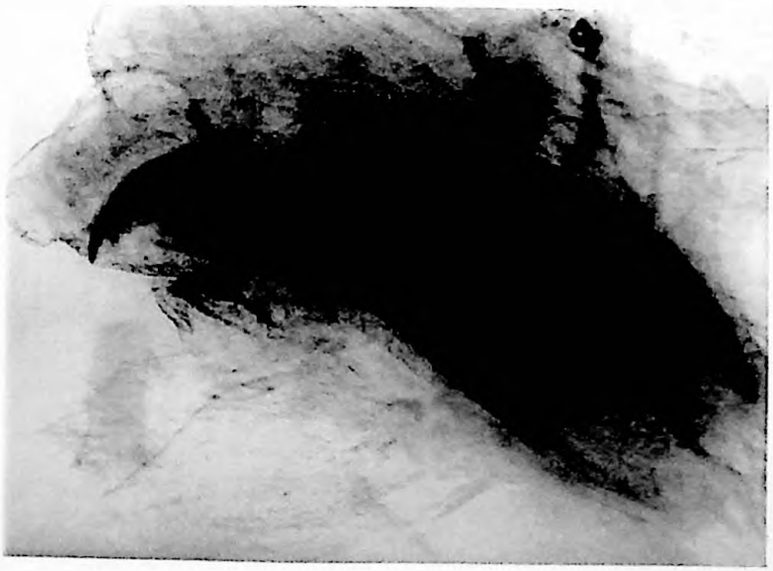


Figure 12. Carrion fly species observed in sunlit (Y) and shaded (D) conditions for Experimental Periods I through IV.

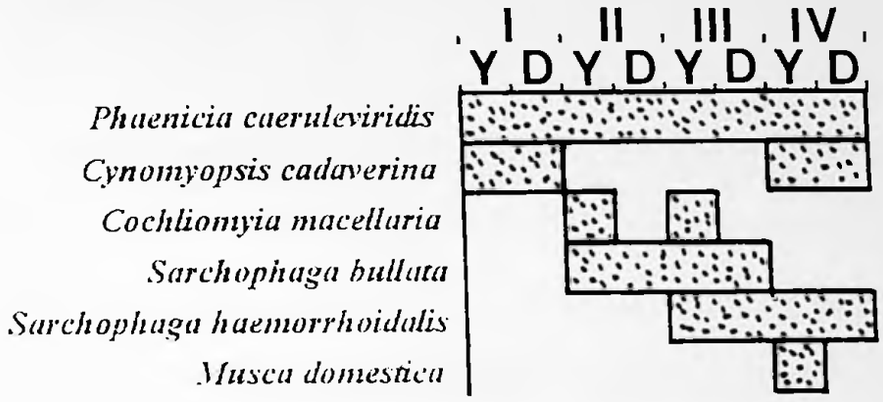

I II III IV
Y D Y D Y D Y D
Phaenicia caeruleviridis
Cynomyopsis cadaverina
Cochliomyia macellaria
Sarchophaga bullata
Sarchophaga haemorrhoidalis
Musca domestica

Figure 13. Various life cycle stages of carrion flies, indicated by stippled bars, observed during Experimental Period I (March/Apr) at sunlit and shaded experimental plots. Ccad = *Cynomyopsis cadaverina*; Pcae = *Phaenicia caeruleiviridis*. Life cycle stages are: adults (a); eggs (e); first, second, and third stage (1, 2, and 3) larvae. Daily temperatures for Experimental Periods are shown above figure. A & B—*C. cadaverina* associated with sunlit and shaded carrion, respectively; and C & D—*P. caeruleiviridis* associated with sunlit and shaded carrion, respectively.

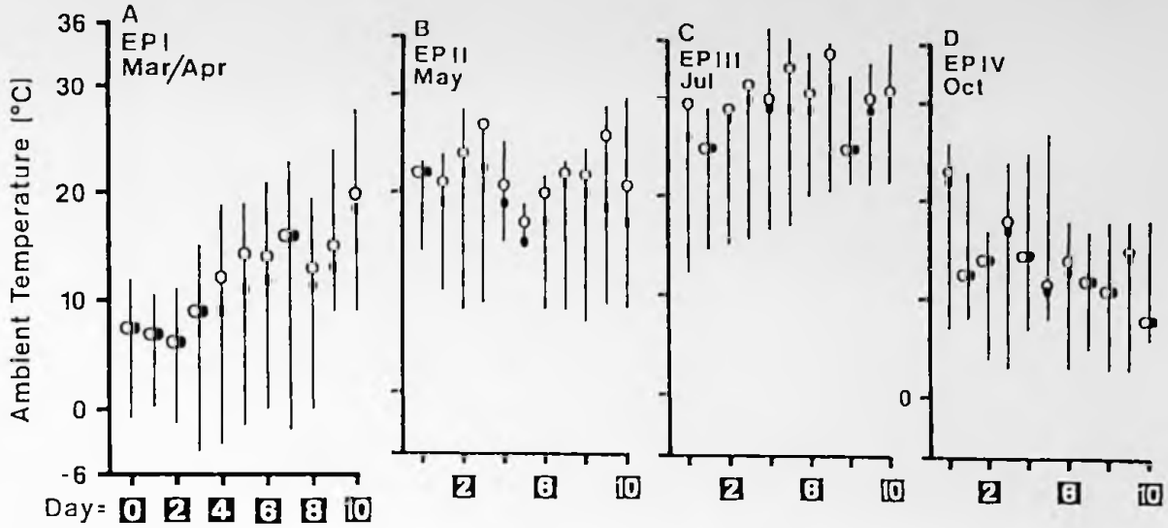

A
EPI
Mar/Apr
B
EPII
May
C
EPIII
Jul
D
EPIV
Oct
Ambient Temperature [°C]
36
30
20
10
0
-6
Day= 0 2 4 6 8 10
2 8 10
2 8 10
2 8 10
0

Fig. 13

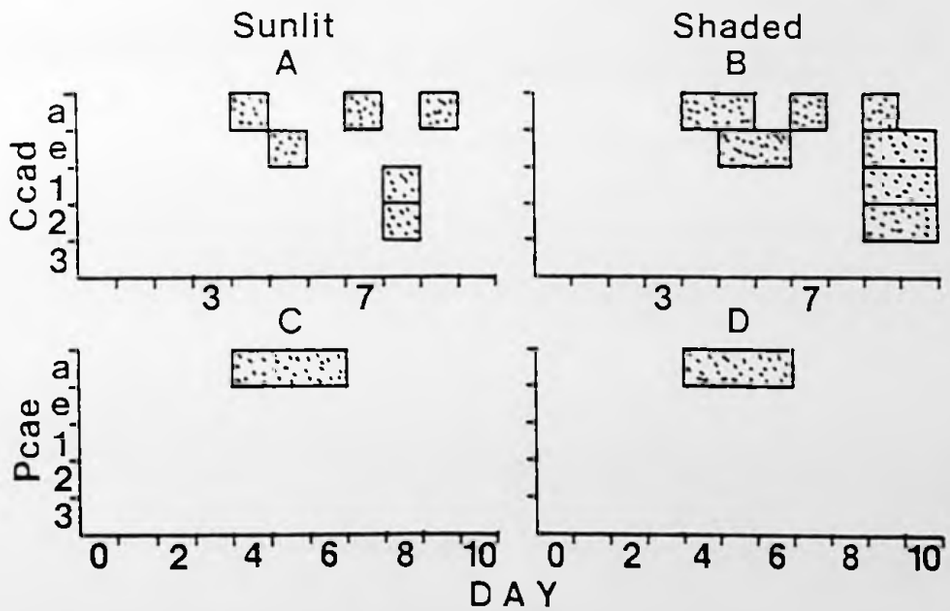

Sunlit
A
Shaded
B
Ccad
a
e
1
2
3
3 7
3 7
C
D
Pcae
a
e
1
2
3
0 2 4 6 8 10
0 2 4 6 8 10
DAY

Figure 14. Various life cycle stages of carrion flies, indicated by stippled bars, observed during Experimental Period II (May) at sunlit and shaded experimental plots. Pcae = *Phaenicia caeruleiviridis*; Cmac = *Cochliomyia macellaria*; Sbul = *Sarcophaga bullata*. Life cycle stages are: adults (a); eggs (e); first, second, and third stage (1, 2, and 3) larvae. Daily temperatures for Experimental Periods are shown above figure. A & B--*P. caeruleiviridis* associated with sunlit and shaded carrion, respectively; C & D—*C. macellaria* associated with sunlit and shaded carrion, respectively; and E & F—*S. bullata* associated with sunlit and shaded carrion, respectively.

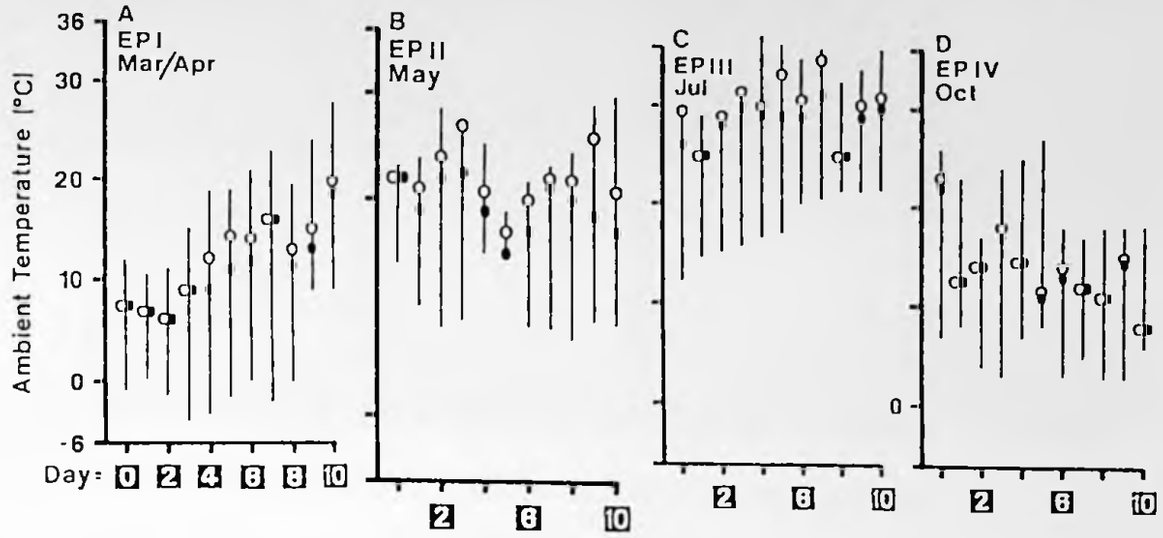

A
EP I
Mar/Apr
B
EP II
May
C
EP III
Jul
D
EP IV
Oct
Ambient Temperature [°C]
36
30
20
10
0
-6
Day: 0 2 4 6 8 10
2 6 10
2 6 10
2 6 10

Fig. 14

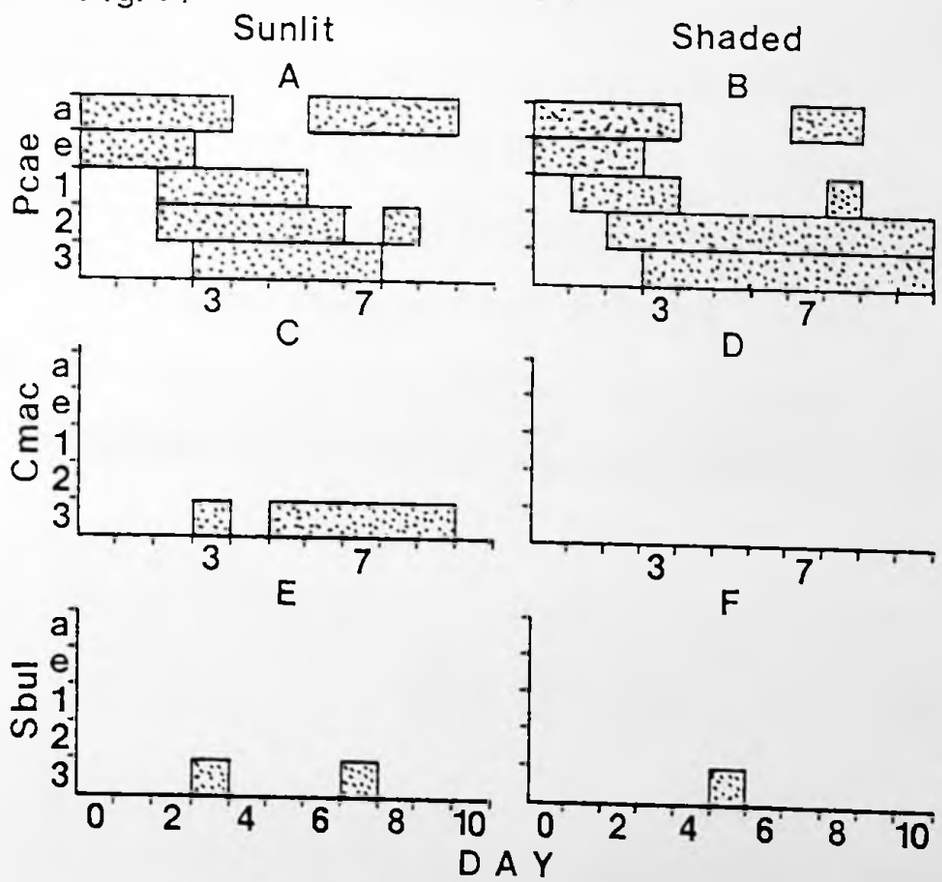

Sunlit
Shaded
A
B
C
D
E
F
Pcae
Cmac
Sbul
a
e
1
2
3
3 7
0 2 4 6 8 10
DAY

Figure 15. Various life cycle stages of carrion flies, indicated by stippled bars, observed during Experimental Period III (Jul) at sunlit and shaded experimental plots. Pcae = *Phaenicia caeruleiviridis*; Cmac = *Cochliomyia macellaria*; Sbul = *Sarcophaga bullata*; Shae = *Sarcophaga haemorrhoidalis*. Life cycle stages are: adults (a); eggs (e); first, second, and third stage (1, 2, and 3) larvae. Daily temperatures for Experimental Periods are shown above figure. A & B—*P. caeruleiviridis* associated with sunlit and shaded carrion, respectively; C & D—*C. macellaria* associated with sunlit and shaded carrion, respectively; E & F—*S. bullata* associated with sunlit and shaded carrion, respectively; and G & H—*S. haemorrhoidalis* associated with sunlit and shaded carrion, respectively.

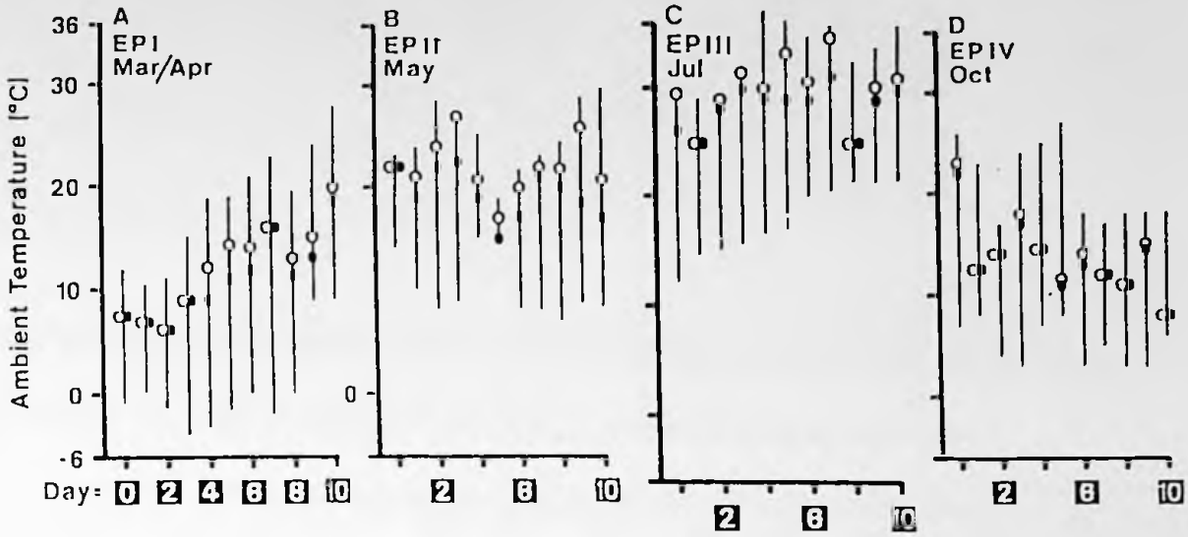

A
EPI
Mar/Apr
B
EPII
May
C
EPIII
Jul
D
EPIV
Oct
Ambient Temperature [°C]
36
30
20
10
0
-6
Day= 0 2 4 6 8 10
2 6 10
0
2 6 10
2 6 10

Fig. 15

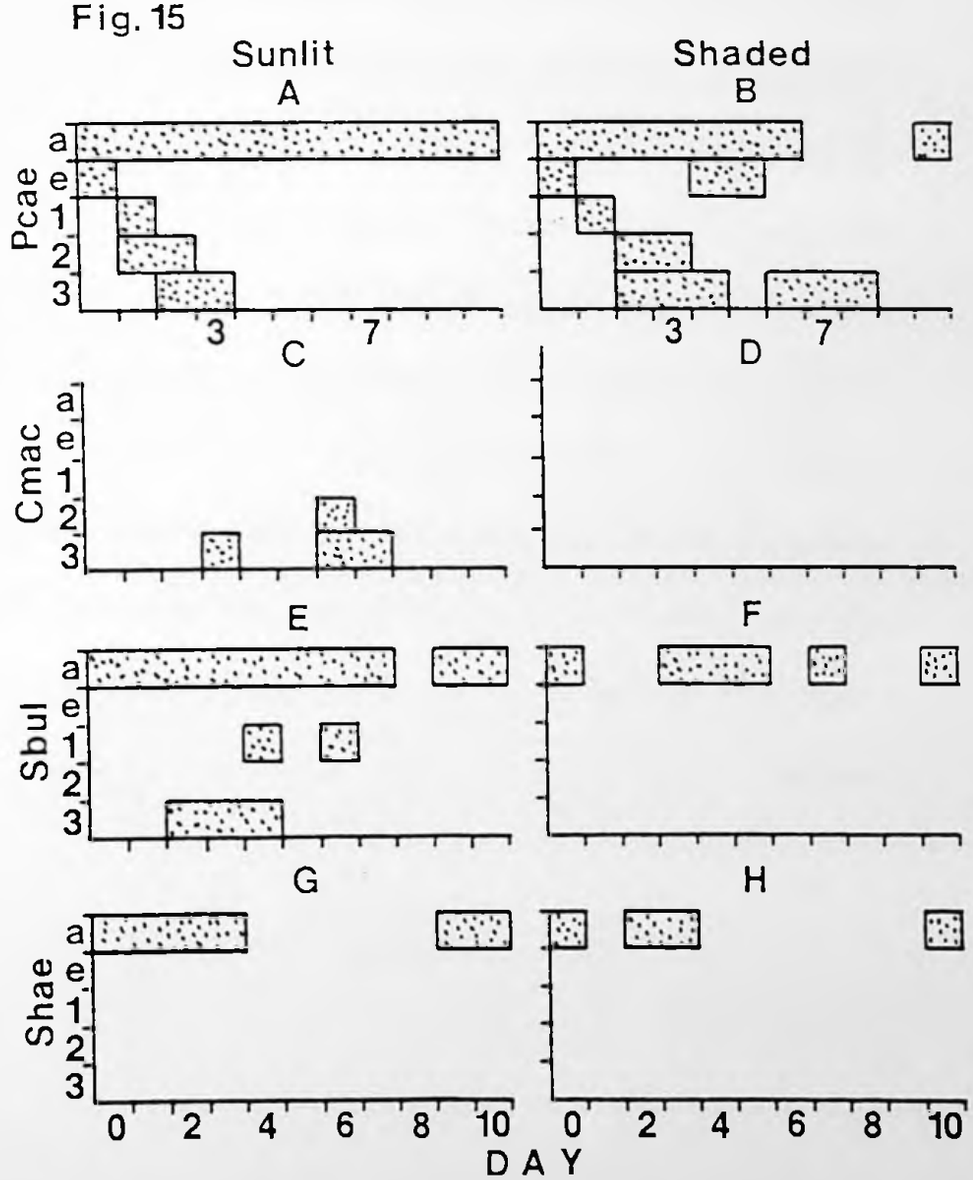

Sunlit
Shaded
A
B
Pcae
a
e
1
2
3
3
7
C
D
Cmac
E
F
Sbul
G
H
Shae
0 2 4 6 8 10
DAY

Figure 16. Various life cycle stages of carrion flies, indicated by stippled bars, observed during Experimental Period IV (Oct) at sunlit and shaded experimental plots. Pcae = *Phaenicia caeruleiviridis*; Ccad = *Cynomyopsis cadaverina*; Shae = *Sarcophaga haemorrhoidalis*; Mdom = *Musca domestica*. Life cycle stages are: adults (a); eggs (e); first, second, and third stage (1, 2, and 3) larvae. Daily temperatures for Experimental Periods are shown above figure. A & B—*P. caeruleiviridis* associated with sunlit and shaded carrion, respectively; C & D—*C. cadaverina* associated with sunlit and shaded carrion, respectively; E & F—*S. haemorrhoidalis* associated with sunlit and shaded carrion, respectively; and G & H--*M. domestica* associated with sunlit and shaded carrion, respectively. Open, dashed bars represent presence of life cycle stages of either *P. caeruleiviridis* or *C. cadaverina*, as there is no way of distinguishing between the two fly species.

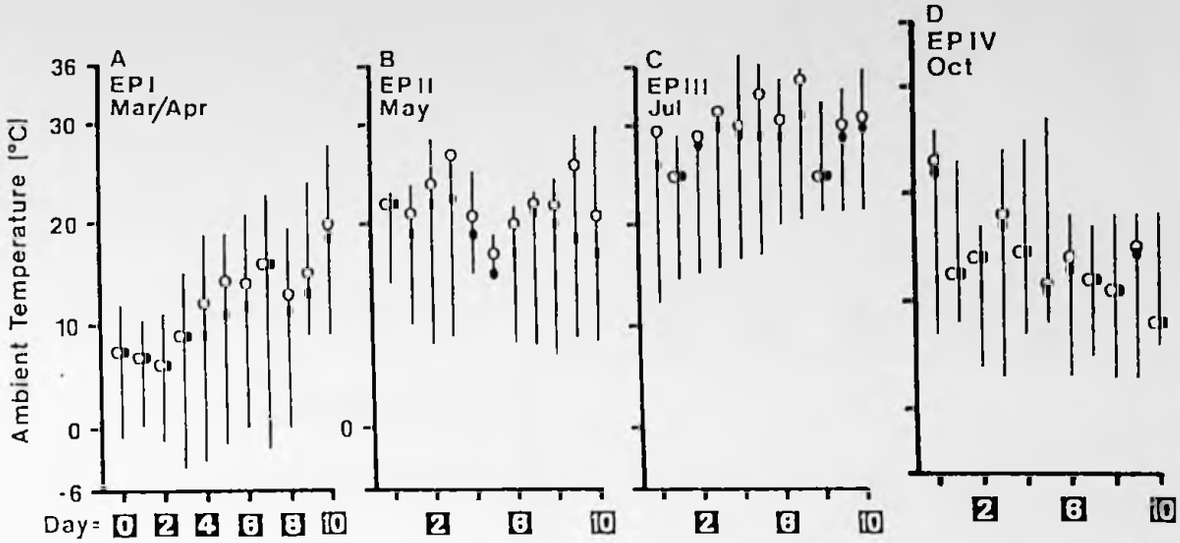

A
EP I
Mar/Apr
B
EP II
May
C
EP III
Jul
D
EP IV
Oct
Ambient Temperature [°C]
36
30
20
10
0
-6
Day= 0 2 4 6 8 10
0
2 6 10
2 6 10
2 6 10

Fig.16

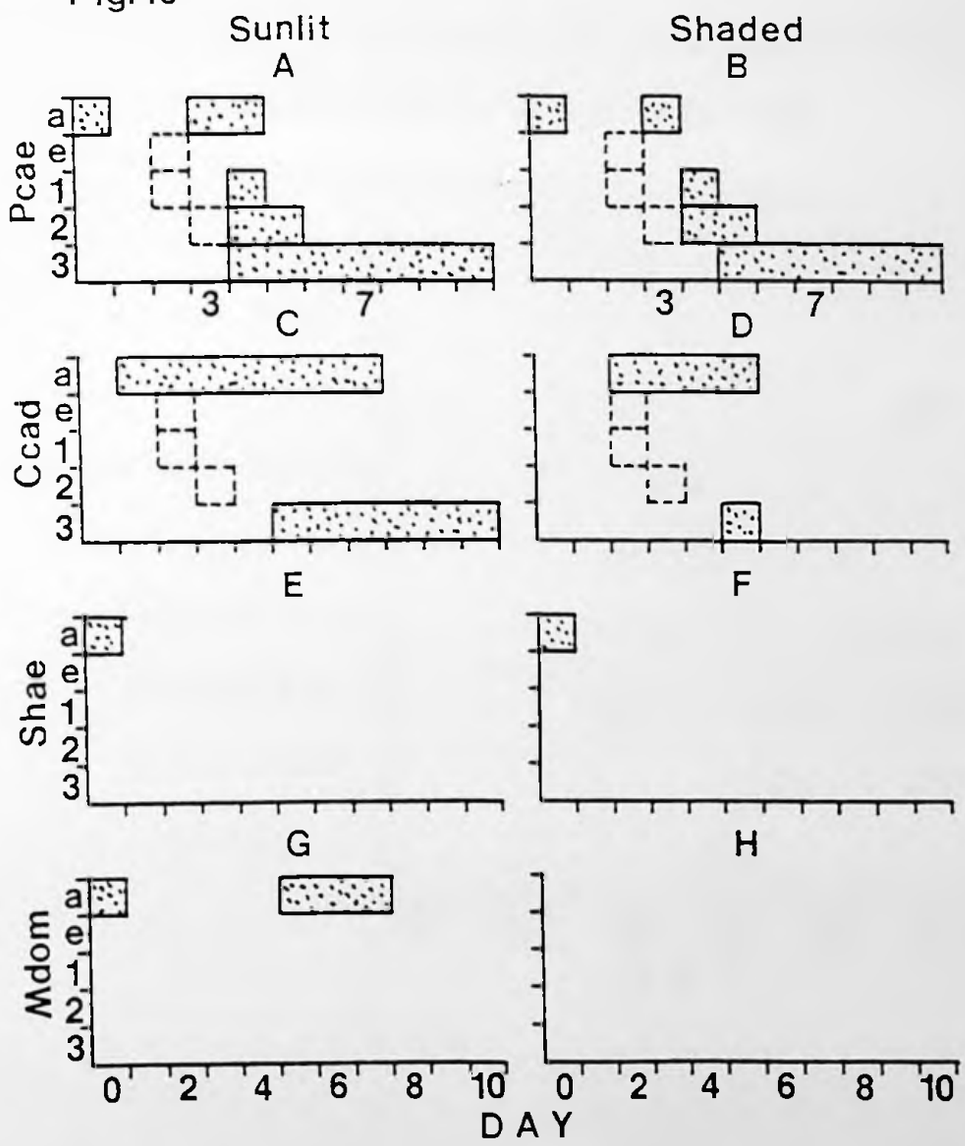

Sunlit
Shaded
A
B
C
D
E
F
G
H
Pcae
Ccad
Shae
Mdom
a
e
1
2
3
3
7
0 2 4 6 8 10
DAY

Figure 17. Daily presence of carrion beetle species (plus one hemipteran) associated with sunlit (open bars) and shaded (solid bars) rat carcasses in Experimental Period II (19 May through 29 May, 1999).

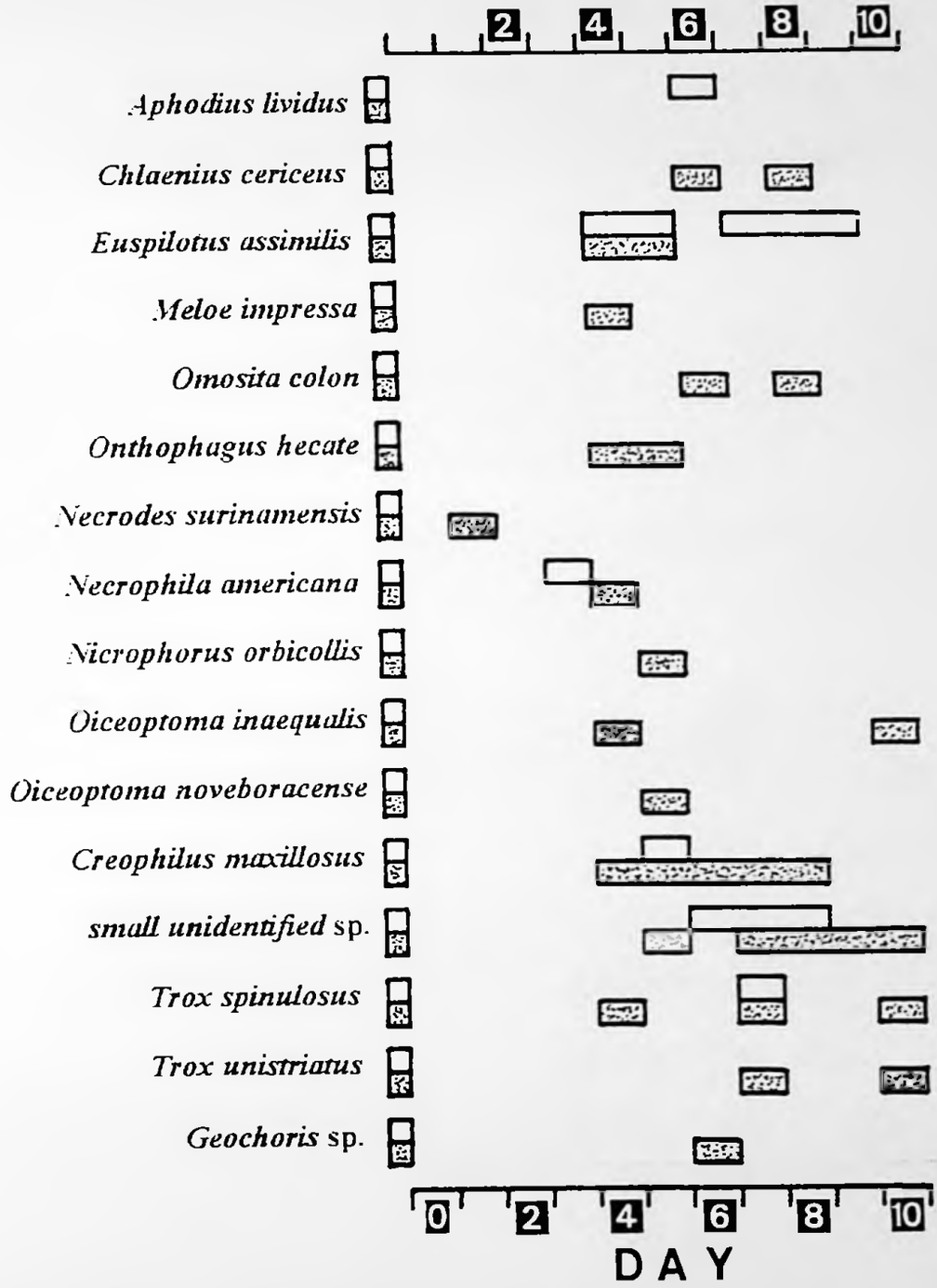

2
4
6
8
10
Aphodius lividus
Chlaenius cericeus
Euspilotus assimilis
Meloe impressa
Omosita colon
Onthophagus hecate
Necrodes surinamensis
Necrophila americana
Nicrophorus orbicollis
Oiceoptoma inaequalis
Oiceoptoma noveboracense
Creophilus maxillosus
small unidentified sp.
Trox spinulosus
Trox unistriatus
Geochoris sp.
0
2
4
6
8
10
DAY

Figure 18. Daily presence of carrion beetle species associated with sunlit (open bars) and shaded (solid bars) rat carcasses in Experimental Period III (12 Jul through 22 Jul, 1999).

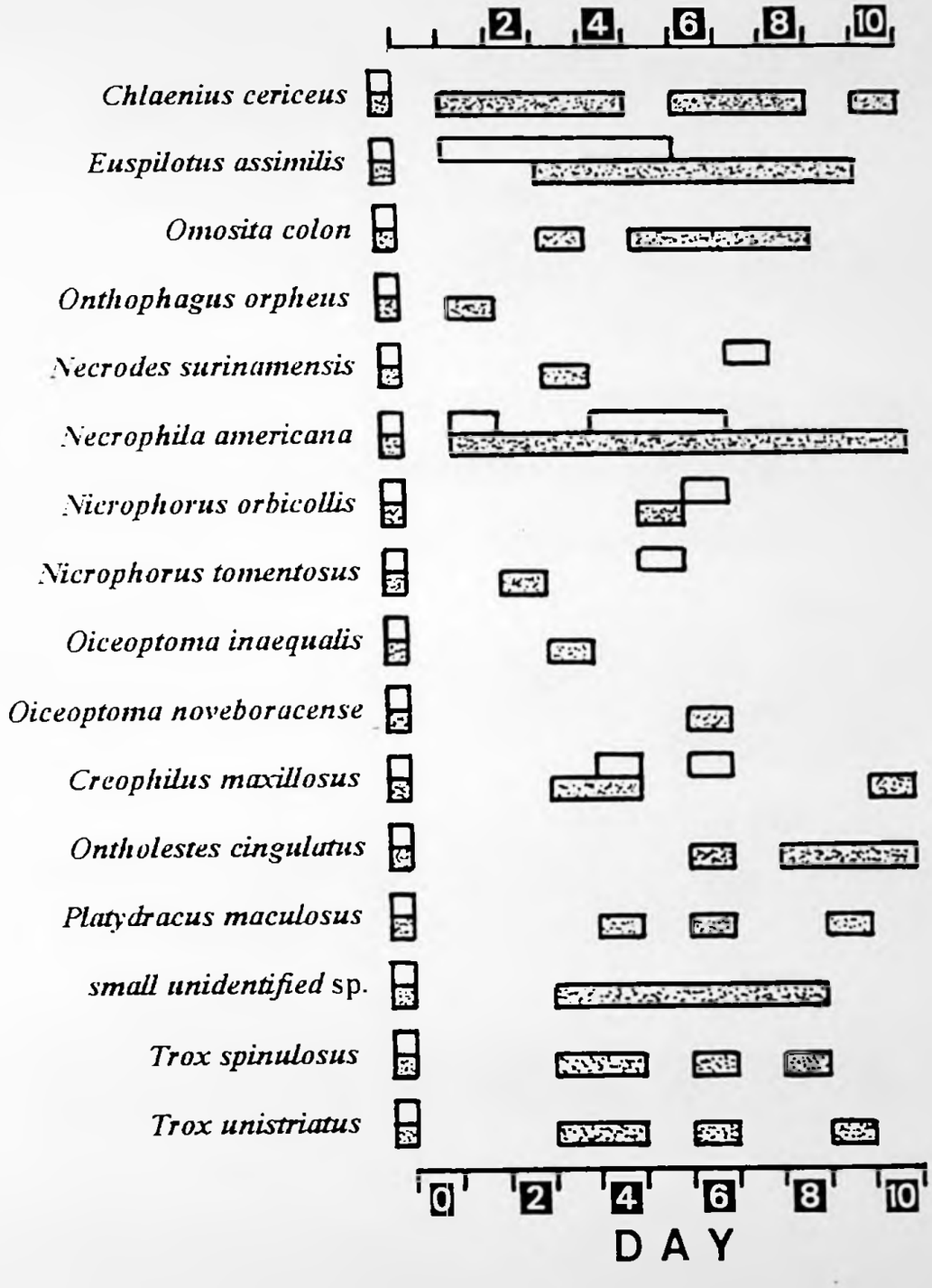

2
4
6
8
10
Chlaenius cericeus
Euspilotus assimilis
Omosita colon
Onthophagus orpheus
Necrodes surinamensis
Necrophila americana
Nicrophorus orbicollis
Nicrophorus tomentosus
Oiceoptoma inaequalis
Oiceoptoma noveboracense
Creophilus maxillosus
Ontholestes cingulatus
Platydracus maculosus
small unidentified sp.
Trox spinulosus
Trox unistriatus
0
2
4
6
8
10
DAY

Figure 19. Daily presence of carrion beetle species associated with sunlit (open bars) and shaded (solid bars) rat carcasses in Experimental Period IV (13 Oct through 23 Oct, 1999).

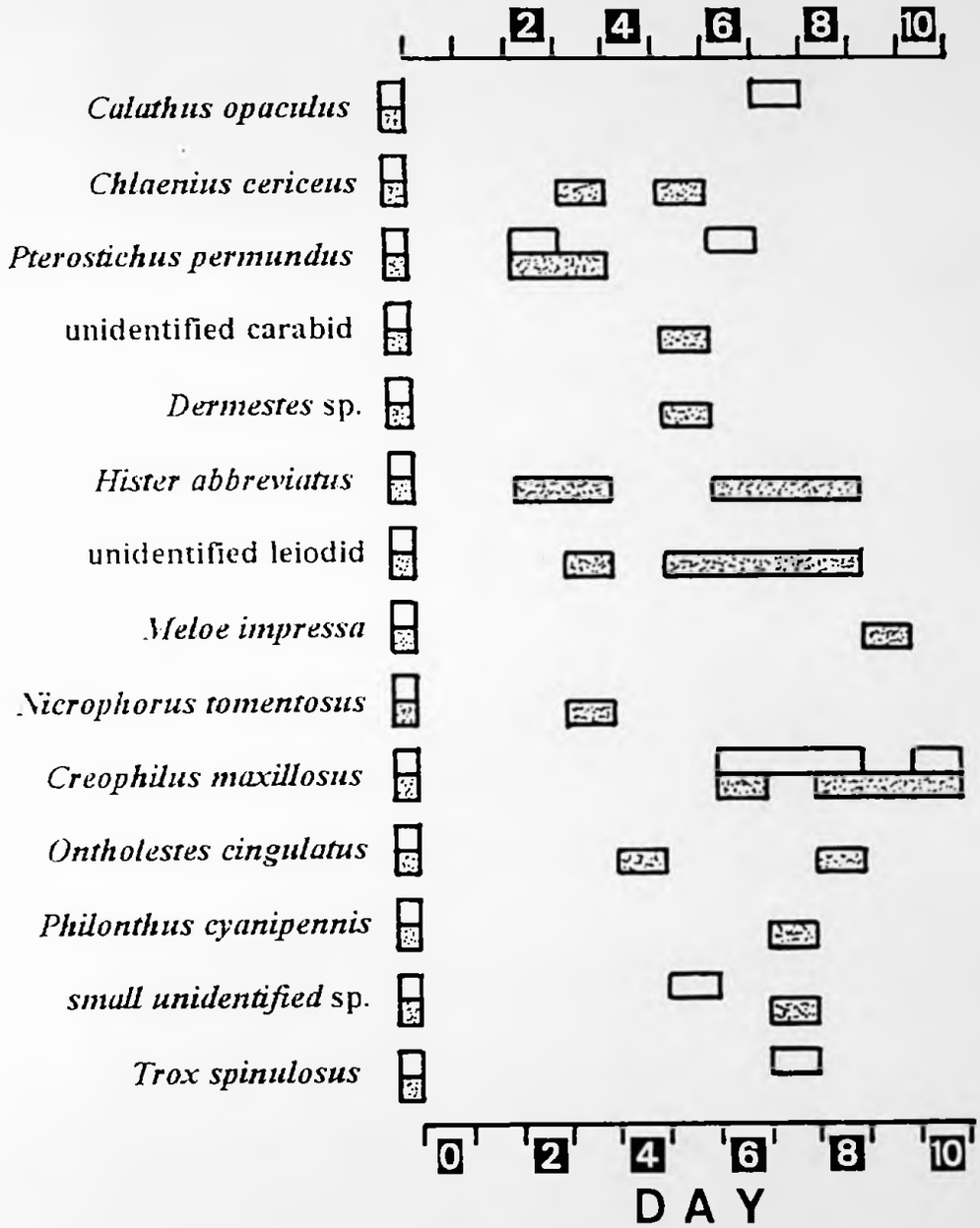

2
4
6
8
10
Calathus opaculus
Chlaenius cericeus
Pterostichus permundus
unidentified carabid
Dermestes sp.
Hister abbreviatus
unidentified leiodid
Meloe impressa
Nicrophorus tomentosus
Creophilus maxillosus
Ontholestes cingulatus
Philonthus cyanipennis
small unidentified sp.
Trox spinulosus
0
2
4
6
8
10
DAY

Figure 20. A. Slug individuals invading ventral, abdominal incision.

B. Slug individual invading bodily orifice (ear).

C. Slug individual invading bodily orifice (anus).

A

B

C

Appendix I

DEPARTMENT OF THE ARMY
HUNTINGTON DISTRICT, CORPS OF ENGINEERS
502 EIGHTH STREET
HUNTINGTON, WEST VIRGINIA 25701-2070

REPLY TO
ATTENTION OF:

MEMORANDUM OF UNDERSTANDING OF GREENBOTTOM

1. The principal purpose of the U.S. Army Corps of Engineers' ownership of an 836-acre site known as Glenwood Bend, is to mitigate for lost wildlife habitat at the Gallipolis Locks replacmeent construction site.

2. The West Virginia Department of Natural Resources has leased the Glenwood Bend site from the U.S. Army Corps of Engineers and is responsible for wildlife and habitat management, public safety, protection of historic and prehistoric features, and managing public interpretation of the area.

3. All parties agree in principle that the historic Jenkins House located on the site and the property immediately surrounding it, as mutually agreed by the undersigned, should be available for public interpretation. This can be best accomplished by the West Virginia Department of Culture and History. Such property shall consist of approximately four acres immediately surrounding the Jenkins House under the management of Culture and History. The Department of Natural Resources will manage an additional non-hunting area surrounding the four acres. That area also will be available for programming by Culture and History in concert with the Department of Natural Resources.

a. Subject to funding, the Department of Culture and History agrees to explore the possibility of subleasing the Jenkins House from the Department of Natural Resources with the purpose of restoring the property to an appropriate historic period and making it available for public interpretation and programming.

b. The Department of Culture and History will provide a plan for the management of the Jenkins home through the Department of Natural Resources to the Corps of Engineers for approval. This plan will identify who will occupy the property and to what degree intitial public use can be made available. The plan also will provide a guideline for eventual restoration and full public use of the home after rehabilitation by the Corps. The plan will be subject to approval by the signers of the Memorandum of Agreement on the historic properties at the Glenwood Bend mitigation site. Signers are the National Advisory Council on Historic Preservation, the U.S. Army Corps of Engineers and the West Virginia State Historic Preservation Officer.

Appendix I (continued)

c. If the Department of Culture and History does not have the initial management plan approved when occupancy is available, the Department of Natural Resources will occupy and protect the property until a satisfactory management plan is available.

4. The Department of Natural Resources will ensure that the Jenkins House will be immediately occupied when available.

J. EDWARD HAMRICK III
Director of Department
of Natural Resources

NORMAN L. FAGAN
Commissioner of
Culture and History

THOMAS E. FAREWELL
Colonel, Corps of
Engineers
District Engineer